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Contents of previous issues of the JOURNAL OF ANIMAL SCIENCE are indexed in the Agricultural Index, which is available in most libraries.

A REVIEW OF BLOAT IN RUMINANTS[1]

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MANY recent papers have referred to the literature bearing upon certain aspects, but no one heretofore has reviewed all phases of ruminal physiology and pathology relating to the bloat problem. For the purposes of acquainting workers with all phases of the problem and revealing the gaps in our present knowledge, such a review would seem desirable and is being attempted in the present article.

Cattle, sheep, goats, and probably all other ruminants are subject to bloat. To our knowledge no one has attempted to determine experimentally the relative susceptibility of different species to this condition. From a survey in Scotland, McCandlish (121) obtained evidence that dairy cows were more susceptible than beef animals or sheep. Mead, Cole and Regan (125) noted that the feeding of green alfalfa tops caused less serious bloat in sheep than in dairy cows, Since, however, they compared only four cows and four sheep, the difference could easily be explained on the basis of individual susceptibility rather than upon a species difference. According to Bell and Britton (26), fat lambs were more susceptible to bloat on Ladino clover than unfinished lambs.

Seriousness of the Bloat Problem[6]

Merely determining actual losses attributable to bloat is not a satisfactory means of evaluating the seriousness of the bloat problem. Expensive procedures followed to prevent bloat, as well as restrictions on the use of legumes because of danger from bloat, should also be considered. For example, in certain irrigated sections alfalfa is cut green and fed in the feedlot because, in this manner, bloat occurring on pasture is avoided. Mead, Cole and Regan (125) have postulated that less bloat is caused by feeding cut green alfalfa than by pasturing, because in this way the animal is forced to eat the entire plant, not merely the succulent tops. In their opinion, consumption of the coarser stems stimulates belching. When only the tops were

[1] Fourth Report of the Committee on Animal Health of the National Research Council. Sponsored by a Subcommittee on the Prevention of Bloat consisting of H. H. Cole, Chairman, R. S. Amadon, R. W. Dougherty, C. F. Huffman, T. M. Olson and A. F. Schalk.

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[6] This section was prepared by A. F. Schalk.

cut and fed in the feedlot, serious bloat was easily induced—a fact that supports this view.

Frequently animals on legume pasture are supplemented with hay for the sole purpose of preventing bloat. This is an added cost. Another expensive procedure is to keep watch while animals are pasturing legumes so that bloated animals may be promptly removed. According to McIntosh (122), not only was this practice used in Canada, but some herds were sold because of difficulty with bloat. Still others restrict the pasturing period on legumes. In most instances this restriction necessitates costly supplemental feeding. One steer feeder in Nebraska (personal communication) reported that he had abandoned the practice of pasturing alfalfa because of losses from bloat. Another serious aspect of the problem is that when conditions are favorable for bloat the losses of individual stockmen may become catastrophic. One dairyman (8) reported, for example, that, although he had been pasturing alfalfa at various times for two years with no evidence of bloat, after a rain 12 of 17 cows bloated and 3 died before the veterinarian arrived. These animals had not been on pasture for more than an hour.

Very few surveys of the losses attributable to bloat have been made. Welch, Marsh and Tunnicliff (206) state, on the basis of a survey in Montana, that cattlemen may expect a loss from bloat on sweet-clover pasture of less than 1 percent and sheepmen about 0.5 percent. McCandlish (121) found, from replies of 152 livestock producers in Scotland, that bloat was prevalent on 34 percent of farms with dairy cattle. Hanson and Boyd (69) made a survey of 1,106 herds with nearly 30,000 head of cattle in Minnesota. A total of 11,205 head were pastured on sweet clover and alfalfa in approximately equal numbers. From May to October, 182 cases of bloat were reported; and of these 50 were fatal. Alafalfa caused 28 deaths; sweet clover 20. Six cases of bloat (none fatal) were reported on nonlegume pasture. Only 25 animals under 2 years of age bloated.

In California, Bell and Britton (26) made (in July and August) a 7-week survey of losses on Ladino clover pasture. These observations were confined to six ranches with a livestock population of 47,000 lambs and 3,200 cattle. Twenty lambs, representing about 50 percent of the total losses during the period, died of bloat. Two steers died from this cause. More deaths from bloat occurred in pastures with a stand of pure clover, or with a very high percentage of clover; and bloat was not a problem in certain pastures covered with more than 50 percent of grasses. Though the number of fields with pure clover involved in these studies was not specified, the same workers state (personal communication) that very few fields consisted exclusively of Ladino.

Conditions under which Bloat Occurs[7]

Acute and Chronic Bloat

Before one can discuss intelligently the conditions under which bloat occurs, bloat must be classified according to cause. Bloat is most frequently classified as "chronic" and "acute." Broadly speaking, acute bloat usually depends upon the nature of the feed, whereas chronic bloat is due to an abnormality in the physiological state of the animal. Since certain feeds such as ground hay and grain (125) can lead to sub-acute bloat of more or less chronic nature the terminology is not altogether satisfactory. As a matter of fact, bloat on green legumes is usually referred to as acute. Lyons (105) in New Zealand, however, reports that cows on legume pasture may remain in a more or less tympanic state for months during certain seasons. The terms will be accepted, however, in this publication because they are well established in the literature. "Chronic" will describe bloat extending over a considerable period and not dependent upon special feed conditions—in other words, bloat resulting from a defective mechanism for eructation. The condition might be due, for example, to a partial obstruction of the esophagus by enlarged mediastinal lymph glands, to a defective nervous mechanism necessary to elicit the eructation reflex, or to atony of the muscles involved in eructation.

In this review, the term "acute" will be applied to bloat that occurs because of an abnormal feeding regime. The feeding of tubers may result in choke—an instance of acute bloat due to the size and hardness of ingested food particles. Or the pasturing of cows on legumes may lead to bloat in a few hours. Bloat on green legumes may fall into two categories: one in which free gas is present, and another in which the gas is incorporated in the ingesta as a frothy mass. This point will be discussed at greater length in a later section. According to findings of Mead, Cole and Regan (125), bloat will continue to occur over long periods if the same type of pasture is used. In this review, nevertheless, such bloat will be classified as acute.

Mead and Goss (127) have reported that bloat will occur on grain alone—a condition not entirely comparable with bloat on green legumes, since it occurs only after ruminants have been maintained on such diets for long periods (Cole and Mead, unpublished data). These authors presume that one cause of bloat in such instances is the lack of coarse irritating material; in this respect the trouble is comparable with fresh legume bloat. They think, however, that a second factor is involved (for example, ruminal atony) which develops only over a considerable period of time. When established,

[7] This section prepared by A. F. Schalk.

this bloat is usually chronic; but since it depends upon a certain feeding regime, it is here placed in the category of acute bloat. When the condition is defined in this manner, the relation of feed to bloat can be discussed more intelligently: the definition recognizes that chronic bloat may occur on any feed.

Relation of Bloat to Feed

In nonchronic bloating animals, bloat may appear under certain feed conditions. Legumes in the prebloom stage are most frequently the cause of acute tympany. Bloat has occurred on the following green legumes: alfalfa, Ladino clover, sweet clover, bur clover, red and white clover, trefoil, and peas. It apparently occurs on these plants in all parts of the world adapted to their culture. To mention a few areas, bloat on legumes has been reported in Canada (122), Australia (138), New Zealand (190), India (107), Scotland (121), South Africa (157), England (25), Germany (50), and the United States (206).

Many other feeds have been reported as causing the condition. Eber (50) reports bloat in cattle fed cabbage leaves. According to McCandlish (119) one of two calves fed on milk as the sole ration bloated for several days before death. Mead and Goss (127) have reported bloat in animals fed on grain alone. Since, however, bloat on milk or grain developed only after a long period on the particular ration, a physiological change in the animal must have occurred. For this reason, the bloat on these feeds belongs in a different category from bloat on green legumes, which may occur the first day and which does not depend upon a long conditioning period. Perhaps bloat on a watery gruel, mentioned by Begg (25), falls into the same category. Mead, Cole and Regan (125) showed that bloat occurred (in a mild form) on finely ground alfalfa hay and grain, but not on whole alfalfa and grain.

Cattlemen encounter some bloat in feeder cattle, especially during the finishing-off period. Kick, Gerlaugh and Schalk (93) made rather extensive observations on this problem in a number of rumen-fistula steers. In good feeding practices, thin steers, just off the range and entering the feedlot, are given a ration of practically all forage. In time the concentrate (corn) is increased until it predominates up to 4 to 1 over the forage. Shortly after the 50-50 balance is reached, in some animals earlier, a whitish, foamy, frothy mass begins to develop over the ingesta. This mass or layer consists of myriads of very small gas bubbles. The formation of such gas is not uniform in all animals. Probably a corollary exists in accordance with appetite, and with rate and amount of corn consumed. Notwithstanding, the condition becomes aggravated as the corn level mounts. It usually manifests itself 1½ to 3 hours after feeding, when the animals begin to bulge in the left flank region.

As the foamy gas mounts in volume the pressure increases; some animals exhibit considerable uneasiness and lie prone upon the ground, grunting and groaning for some time thereafter.

Obviously, the belching mechanism is not functioning properly in these animals. The evidence at hand does not indicate whether the rumen gas is entrapped as bubbles (foamy bloat) or whether there is simply insufficient coarse roughage in the diet to elicit the eructation reflex. Observation of the foamy layer in rumen-fistula animals favors the first alternative, but the intact animals were not stuck to determine whether free gas would escape.

The feeding of tuberous crops such as potatoes and beets may result in bloat. Some believe that spoiled potatoes are especially dangerous in this respect. The literature does not make clear as to whether the tubers are ground or chopped. It is well recognized that chopped tubers produce bloat by choking. A rancher in California states that he may have 25 cases of "choke" every fall while pasturing 200 head of steers on beet tops. Bloat from choke is like chronic bloat produced by hyperplasia of the mediastinal lymph glands, in that the esophagus is obstructed. In the former, however, eructation is prevented completely; in the latter, only partially.

As a rule there is little difficulty with bloat when animals are pastured on grasses. Taylor in New Zealand (190) states that bloat may occur on rye grasses that have been stimulated to grow rapidly, and Ohman in Australia (138) reports that rapidly growing cereal crops may produce the trouble. In the United States there are apparently no well-authenticated cases of bloat on a pasture consisting solely of grasses. On the contrary, it is widely recognized that bloat may be lessened by mixing grasses with legumes.

Relation between Environment and Bloat

Because bloat so often occurs unexpectedly and under feed conditions which seem normal to the livestock producer, environment has been considered significant. Wetting of legumes by dew or rain is most often regarded as an important etiological factor. This theory has not been disproved, but bloat certainly may occur even on legume pastures that are dry. According to McIntosh (122), much difficulty was experienced in Canada during a period of drought, and bloat occurred irrespective of the amount of moisture on the alfalfa. This report of bloat during a period of drought is in exact contrast to most findings. The severity of the drought probably killed all plants except the alfalfa. Further, during so dry a period there would no doubt be a greater tendency to pasture the alfalfa while young because of the scarcity of feed. Perhaps one hears of bloat under these conditions so rarely because drought of such magnitude is not often encountered. Cole, Mead and Regan (36) also report that bloat may occur on legumes free of

external moisture. McCandlish (121) stated further that many farmers reported bloat during clear weather.

As mentioned above, the amount of rainfall may affect the incidence of bloat by controlling the type of plants that will grow. In the instance discussed, it was assumed that dry weather favored the growth of alfalfa over that of grasses and other plants. Wet weather, on the contrary, seems to favor the growth of white clover: according to Healy and Nutter (72), excessive rain has sometimes resulted in a preponderance of white clover over blue grass, with consequent severe difficulty with bloat. Pammel (148) attributed an unusual amount of bloat on sweet clover in Iowa to excessive growth of sweet clover because of high rainfall.

Schalk (unpublished data) relates an experience that bears on the role of humidity in bloat. The first twenty years of his life were spent on a large dairy farm in southern Ohio where volunteer blue grass was practically the only pasture available. During this time there were three distinct periods of three to four successive years in which the spring rainfall was considerably above normal. In each of these periods white clover practically replaced the otherwise pure culture of blue grass, and this predominance of white clover was attended by a serious bloat problem. On the other hand, when the weather conditions returned to normal and the pasture reverted to a practically pure culture of blue grass, bloat disappeared. There simply was no bloat in years of ordinary rainfall.

Another experience of Schalk relates to sweet clover. During the late teens and early twenties of the present century, the growing of sweet clover was given a tremendous impetus in the North Central States, particularly in the Red River Valley. Since practically all the agricultural divisions of North Dakota State College were, in one way or another, interested in sweet clover, its evolution was followed closely. In all the years before 1924, no bloat in either cattle or sheep had been reported to interested divisions. Evidently the condition did not exist, or at least was extremely rare.

Almost over night, the situation changed. Beginning about June 15, 1924, and through the next 15 or 20 days, the Veterinary Division alone heard from more than 60 different farms where acute bloat on sweet-clover pasture prevailed in cattle or sheep or both. Often many animals were stricken, with accompanying great economic losses from deaths.

The weather conditions that spring deserve comment and, no doubt, were truly significant. Beginning about May 15, or a little later, there were consistent periodic showers about every 3 or 4 days. The rains were attended with more than the usual amount of sunshine and warm weather. As a result, the sweet clover grew rapidly and luxuriantly and was extremely suc-

culent. In Schalk's opinion the unusual moisture, together with the warm weather caused the bloat indirectly by promoting plant growth.

Evidently, however, the role of humidity is still imperfectly understood. The effect of the amount of rainfall upon the nature of the plant growth is perhaps very important. In the opinion of many, the moisture in the plants has a more direct effect. It could influence the rapidity of food consumption by virtue of a change in palatability produced by its presence; or, less likely, it might influence the rate of fermentation within the rumen. The present writers consider the latter less likely because the rumen normally contains a copious amount of water, which would not be markedly increased by the small amount consumed with the plant.

Many regard frost and wind as important etiological factors. Evidence in favor of these views is not convincing. After more important questions have been settled, some attention might be given to these possibilities.

Sources of Rumen Gases[8]

Composition of Rumen Gases

Apparently the first analysis of gas given off from the rumen content was published by Popoff (151) working in Hoppe-Seyler's laboratory. He found: 32% CO_2; 6% H_2; 1% CH_4; and 60% N_2. Quite different is the result of Tappeiner (189), who recognized cellulose digestion in the ruminant as a fermentation process and reported the following composition for gases taken from the rumen: 65% CO_2+H_2S; 31% CH_4; 0.2% H_2; and 4% N_2. The most recent analyses (Kleiber, Cole and Mead, 94) average to practically the same result for rumen gas taken from cows on alfalfa pasture, namely: 67% CO_2; 26% CH_4; 7% N_2+H_2; 0.1% H_2S; and less than 1% O_2.

Hydrogen has been detected in most cases where volumetric gas analysis was used—first by Markoff (111) then by Zuntz and others (222).

In laboratories where combustion of respiration gas in an aliquot air stream was used, hydrogen seemed to be absent from rumen gases. Thus Fries (59) found that the atomic ratio of H:C in the combustion products of gas from steers was exactly that in methane; he decided that methane was the only combustible gas given off by the ruminant. Armsby and Fries (12) repeated this observation; and it was recently confirmed by Lugg (104), who noted that the combustible part of rumen gas from sheep contained hydrogen and carbon atoms in a ratio of 3.999 ± 0.012, and who concluded from this result that the combustible gas given off by sheep was extremely pure methane.

Objection against this conclusion can be raised by the argument that rumen gas may contain higher hydrocarbons or other volatile carbon com-

[8] This section was prepared by Max Kleiber.

pounds with less than 4 hydrogen atoms per carbon atom; this surplus of carbon might just balance the free hydrogen, thus producing the H:C ratio in methane.

On the other hand, the result of the volumetric determination of hydrogen based on the gas contraction after combustion may also involve errors. Thus, as Hoppe-Seyler (78) points out, the combustion of methane in an explosion set off by a spark from a Ruhmkorff inductor may lead to the formation of ozone and other oxidation products, producing a contraction of the gas, which then is interpreted as the result of hydrogen combustion. The modern method of combusting the gas at the surface of a hot platinum wire may avoid this difficulty. Even so, the problem of hydrogen in rumen gas deserves a methodical reinvestigation.

Nitrogen ahd hydrogen sulfide are normal constituents of rumen gas; oxygen is often found; and Dougherty (44) reports the presence of carbon monoxide in concentrations up to 0.17 percent. A thorough check of this last observation by various methods seems desirable. In the present review, the sources of carbon monoxide in rumen gas will not be traced.

Sources of Methane in the Rumen

a. Methane from food. Methane may be regarded as the most typical rumen gas because it is produced in the rumen at much higher rates than in any other part of the ruminant's body.

Popoff (151) recognized cellulose as the source of methane produced in sewer mud. Tappeiner (189), by determination of carbon balances in fermenting rumen content with added cotton or paper, produced evidence that cellulose is a source of methane in the rumen. According to Woodman and Stewart (216), the methane production is not an integral part of cellulose digestion, but rather a side reaction. These authors conclude from fermentation studies at 65° C. that the immediate source of methane, and of other rumen gases, is the sugar resulting from bacterial decomposition of cellulose.

That cellulose is not the only source of methane is indicated by the results of Kellner and Köhler (91) on fattening steers. When either cellulose or starch or sugar (molasses) was added to a maintenance ration, the rate of methane production increased in close correlation to the amount of added carbohydrate. This increase averaged 4.29 grams of methane per 100 grams of digestible carbohydrates added (Kellner, 90).

Armsby and Fries (13) very closely confirmed Kellner's results, finding the production of 4.8 grams of methane per 100 grams of digested carbohydrates, as a mean of 43 experiments with roughages alone; and 4.7 grams as a mean of 22 experiments with rations mixed of roughages and concentrates.

Kleiber, Regan and Mead (in press, Hilgardia) noted that daily methane

production of dairy cows increased when glucose was added to a maintenance ration of Sudan hay. This increase averaged 1.8 grams of methane per 100 grams of added glucose in one series and 2.5 grams in another series of respiration trials. The mean methane production from Sudan hay amounted to 3.5 grams per 100 grams of digested carbohydrates in the hay. Glucose as a source of methane in the rumen, however, deserves further investigation, since Mitchell, Hamilton and Haines (129) observed no increase in methane production when they added glucose to a basic diet for steers.

Kellner found no increase in methane production after the addition of protein (wheat gluten) or fat (peanut oil) to a maintenance ration for steers. Judging from this result, the major if not the only source of rumen methane is carbohydrates, even though Kluyver (97) suggests that the great majority of organic compounds may be capable of being fermented to methane and carbon dioxide. Bach and Sierp (15) actually observed that the microflora of mud produced considerable amounts of methane when meat or egg albumen was used as substrate.

b. Methane from carbon dioxide. The biochemistry of methane formation has recently undergone a rather revolutionary development. Carbon dioxide is the source of methane, according to van Niel's theory represented by the equation

$$4H_2A + CO_2 \rightarrow A + CH_4 + 2H_2O$$

in which *A* represents any organic or inorganic hydrogen acceptor. Since this theory is highly important for the problem of the sources of rumen gases, this review will outline the development leading to this formulation. The account is based mainly on the publications of Barker (to be mentioned).

Söhngen in 1906 (cit. by van Niel, 136) produced evidence that bacteria can reduce carbon dioxide to methane by oxidizing gaseous hydrogen. These results were confirmed later by Fischer, Lieske and Winzer (56) and by Stephenson and Stickland (184).

Van Niel (Barker, 19) then postulated the theory that not only hydrogen gas, but all compounds which can serve as hydrogen donors, are probably capable of supporting the microbiological reduction of carbon dioxide to methane. Barker (20) purified cultures of four species of methane-producing bacteria and confirmed van Niel's theory, especially by analyzing the fermentation of ethyl alcohol to acetic acid. The alcohol was almost quantitatively transformed to acid. This result justified the conclusion that the methane produced during the process originated from carbon dioxide. Direct proof was produced later by using radioactive carbon (24). Barker (21) succeeded in isolating one species of methane-producing microorganisms, namely *Methanobacterium omelianskii*. He showed (22) that this organ-

ism oxidizes ethyl alcohol according to the reaction $2C_2H_5OH + CO_2 \rightarrow 2CH_3COOH + CH_4$, whereby it also produces considerable organic matter (bacterial substance) from carbon dioxide.

Van Niel (136) has reviewed the present knowledge of chemosynthetic carbon dioxide reduction by various organisms and even by animal tissue.

Sources of Carbon Dioxide in the Rumen

a. Carbon dioxide from food. The immediate precursor of carbon dioxide, resulting from fermentation, seems to be either a one-carbon or a three-carbon compound (97); but the number of different organic substances from which microorganisms can produce carbon dioxide is practically unlimited. Since carbon dioxide can disappear from the rumen by reduction to methane, by belching, and by diffusion through the rumen wall, experimental results on rates of carbon dioxide production in the rumen are open to criticism. This criticism applies particularly to Washburn and Brody's (204) calculation of carbon dioxide production rates from the rate of methane production, a calculation based on the assumption that the diffusion rates of these two gases are the same, whereas actually (as will be discussed later) carbon dioxide diffuses more than 20 times as fast as methane.

At present, the only reliable information on rate of carbon dioxide production seems to be obtained from fermentation measurements *in vitro*.

When Markoff (111) added sugar to rumen content fermenting *in vitro*, the rate of carbon dioxide production was greatly increased, and the molar ratio of carbon dioxide to methane in the gas rose from about 4 without sugar to 11 with the addition of sugar. Quin (156) measured the rate of gas production in sheep with rumen fistulas. The nature of the gas evolved was not determined; but according to Markoff's (111) results, which are fully confirmed by Quin's investigation, the assumption appears justified that the major constituent of the gas evolved by Quin's sheep was carbon dioxide. A check on this assumption by future investigations would seem desirable.

Quin's trials indicate two major conditions for the high rate of gas production in the rumen of sheep. 1. Only sugars added to the daily ration, either as pure substance or as constituents of feeds such as alfalfa, produce rapid gas formation during the first 90 minutes after introduction into the rumen; starch or cellulose are ineffective. 2. Rapid gas formation follows the application of sugars only when the rumen contains the necessary microflora—namely, either yeastlike organisms (*Schizosaccharomyces ovis*) that build up glycogen, or iodophylic bacteria that produce starch from sugar by oxidative synthesis.

In starved sheep no rapid fermentation occurs, because the rumen flora is reduced. Quin's later suggestion (157) that animals should be well fed to pre-

vent bloat appears, at first sight, to contradict the observation that rapid fermentation is lacking after starvation.

Even though Quin's experiments indicate that only sugars produce rapid gas formation in the rumen, they do not justify the conclusion that sugars are the only sources of rumen gas—a conclusion hard to reconcile with Kellner's results on methane production from starch and cellulose mentioned above. The emphasis in Quin's results is on "rapid." Cellulose and starch may produce the same amount of rumen gas per gram as sugar, but they require a longer fermentation time.

Markoff (112), in fermentation tests *in vitro*, observed that rumen contents after a ration rich in protein produced more fermentation-carbon dioxide than did rumen contents after a low-protein ration. This observation, though not a strict proof that protein itself is a source of rumen gas, contradicts Kellner's conclusion that only carbohydrates, as additions to a basic ration, increase rumen-gas production. Earlier, Markoff (111) had noted that a filtrate of rumen content that produced 5 cc. of carbon dioxide with cellulose alone produced 10 cc. when asparagin was added, 100 cc. when glucose was added, and over 200 cc. when asparagin and glucose were adeed together. Only modern experiments with tagging of carbon atoms can reveal whether in a strict sense asparagin itself produces carbon dioxide or merely makes sugar produce more.

b. Carbon dioxide from saliva. Saliva contains carbonates corresponding to an equivalent of 200 volumes percent carbon dioxide (112). The rate of saliva secretion of a cow was estimated to 60 liters per day (Colin 1886, cited by McAnally and Phillipson, 118). According to these figures, saliva transports daily an equivalent of 120 liters of carbon dioxide to the rumen.

Saliva, therefore, is a potential source of rumen carbon dioxide that appears rather significant in comparison with the daily rate of methane production, which in a series of recent experiments with dairy cows averaged 180 liters per day in dry cows on maintenance level of food intake and 280 liters per day in lactating cows on production rations (Kleiber, Regan and Mead, in press).

In tests carried out by Markoff (112), the carbon dioxide in the form of carbonates in a fermenting rumen liquid consisting of 10 grams of organic matter amounted to 18 grams, the carbon dioxide from fermentation 35 grams, and the methane production 6.8 grams.

Washburn and Brody (204) noted an unusually high carbon dioxide formation after grass feeding. Assuming that such feeding led to a reduced rate of saliva secretion, they were confronted with the puzzle that carbon dioxide increased even though one of its sources, namely saliva, presumably was decreased. They concluded that "the salivary origin of carbon dioxide may perhaps not be as important as one might expect."

In order to understand better the role of saliva as a source for rumen gas, one should realize that the acidity (pH) in the rumen contents determines whether or not rumen carbon dioxide evolves from carbonates entering the rumen in saliva. Markoff as early as 1911 saw part of this problem correctly: he suggested that saliva (with its alkaline reaction), instead of giving off, may on the contrary absorb carbon dioxide in the rumen.

In the following an attempt is made to define the conditions under which saliva upon entering the rumen absorbs or gives off carbon dioxide and to estimate the extent of these processes.

Cattle seem to have a more alkaline saliva than other domestic animals; a pH of 8.2 has been reported by Schwarte (Dukes, 48). Acidity and carbonate content of solutions such as saliva are correlated; they depend on the partial carbon dioxide pressure of the gas with which these solutions are in contact.

TABLE 1. CARBONATE IN AQUEOUS SOLUTIONS AT VARIOUS pH (THEORETICAL)

1	2	3	4	5	6
pH	Ratio $\frac{CO_3^{--}+HCO_3^-}{H_2CO_3+CO_2}$	"Bound CO_2" $CO_3^{--}+HCO_3^-$ when in contact with gas 1 atmosphere with:		Total CO_2 $CO_3^{--}+HCO_3^-$ plus $H_2CO_3^+ + CO_2$ when in contact with gas at 1 atmosphere with:	
		6% CO_2	70% CO_2	6% CO_2	70% CO_2
		millimol per liter	*millimol per liter*	*millimol per liter*	*millimol per per liter*
8.2	126	176	2142	177.4	2159
8.0	79	111	1343	112.4	1360
7.5	25	35	425	36.4	442
7.0	7.9	11.1	134	12.5	151
6.5	2.5	3.5	42.5	4.9	59.5
6.1	1.0	1.4	17.0	2.8	34.0
6.0	0.79	1.11	13.4	2.5	30.4
5.5	0.25	0.35	4.25	1.8	21.2
5.0	0.079	0.111	1.34	1.5	18.3
4.5	0.025	0.035	0.42	1.43	17.4
4.0	0.0079	0.011	0.13	1.41	17.1
3.5	0.0025	0.0035	0.04	1.40	17.04
3.0	0.00079	0.0011	0.01	1.40	17.01

For a wide range of pH, table 1 gives estimates of total carbonate content, namely "bound CO_2" ($H_2CO_3+CO_2$ dissolved) in solutions which contact expired air (with 6 volumes percent carbon dioxide) or rumen gas (with 70 volumes percent carbon dioxide). The calculation of the table is based on the Henderson-Hasselbalch equation for the relation of "bound" and "free CO_2" and the pH in blood serum, and on Henry's law as follows:

Column 2: From the equation of Henderson and Hasselbalch given in Hitchcock (76):

$$pH = pK + \log \frac{\text{"bound } CO_2\text{"}}{\text{"free } CO_2\text{"}}$$

pK = first dissociation constant of $H_2CO_3 = 6.10$
free CO_2 = molar concentration of undissociated H_2CO_3 plus CO_2
bound CO_2 = total CO_2 minus free CO_2.

Column 3: The "free CO_2" = $H_2CO_3 + CO_2$ for water in contact with gas (at 1 atmosphere pressure) which contains 6 percent CO_2 was obtained by plotting data of Morgan and Maass given by Quin and Jones (160)—moles CO_2 per liter vs. partial pressure. Then, extrapolating for 37° C. of values at 500 mm. pressure and applying Henry's law, we find that 1.4 millimol of "free CO_2" per liter is in equilibrium with the 6 percent gas at 37° C. at 1 atmosphere total pressure. For our calculation, we assume that changes of this equilibrium, by changing concentrations of hydrogen ions or other solutes in the saliva, can be neglected.

Column 4: The same calculation for contact with gas at 1 atmosphere pressure and 70 percent CO_2 leads to 17 millimol "free CO_2" per liter.

Column 5: Column 3 + 1.4.

Column 6: Column 4 + 17.0.

The calculation is presented as a program of what should be measured with saliva (as source of rumen gases), and a rough estimation of the probable results of such measurements, rather than a statement of definite results.

At pH 8.2, saliva in contact with 6 percent carbon dioxide in air is estimated by our calculation to contain per liter 177 millimols of "total carbon dioxide" ($CO_3^{--} + HCO_3^- + H_2CO_3 + CO_2$). This is almost twice the amount reported by Markoff (112) of 200 volumes percent carbon dioxide equivalent in saliva, which figure (90 millimols per liter) according to our calculation more nearly corresponds to a pH of 8.0. Possibly the pH of 8.2 was noted by Markoff when the saliva was in contact with air containing less than 6 percent carbon dioxide.

If saliva containing 112 millimols "total CO_2" per liter at pH 8.0 (see table 1) were brought from contact with expired air (6 percent carbon dioxide) to rumen gas with 70 percent carbon dioxide, each liter of this saliva would abosrb the difference between 1,360 and 112, namely 1,248 millimols (27 liters) of carbon dioxide, provided the pH remained at 8.0. Maintenance of this pH level, however, would require the addition of a great deal of hydroxide to increase the concentration of "bound CO_2." A decrease in the pH of the saliva, as it enters the rumen, is more likely, even if no other liquid were present in the rumen. Assuming the "bound CO_2" in the saliva to remain constant, one may calculate by the Henderson-Hasselbalch equation mentioned above that in saliva flowing from mouth to rumen (containing no other liquid) the pH will shift from 8.0 to 6.91. During this passage each liter of saliva would absorb from the rumen gas only 13 millimols of carbon dioxide to attain a content of 125 millimols "total CO_2" (calculated by interpolation between 151 and 59.5 in the last column of table 1). For the 60 liters

of saliva secreted daily, this absorption would mean a decrease of 17 liters of carbon dioxide in the gas of the rumen. This is a fair estimate of the effect suggested by Markoff.

The only way saliva can become a source of rumen gas is a decrease in pH, below the 6.9 level. Such a shift may occur, for example, by formation of lactic acid in the rumen, a process noted by Phillipson (150).

Smith (177) reports a rather narrow range for pH in rumen contents from Holstein cows with rumen fistulas. In 10 hours after feeding alfalfa hay, the pH of the rumen content dropped from 6.4 to 6.2. During a similar period after feeding alfalfa hay together with molasses beet pulp, the pH decreased from 6.1 to 5.9

Monroe and Perkins (130) report for bovine rumen contents a range of pH from 6.5 to 7.0. They do not state whether their samples were in contact with air or with rumen gas.

The greatest range in pH in rumen contents was observed by Myburgh and Quin (133). These authors sucked samples out of sheep rumen, using permanent fistulas, and determined the pH within a few minutes. They observed average pH levels as high as 7.5 when the sheep were given fresh green alfalfa and as low as 5.7 with a diet of 1 kg. alfalfa hay plus 0.6 kg. crushed maize.

The potential effectiveness of saliva as a source of carbon dioxide in rumen gas may be estimated from this latter figure. An interpolation from the last column of table 1 indicates that at a pH of 5.7 and in contact with 70 percent carbon dioxide gas one liter of saliva would contain 23 millimols "total CO_2." Each liter of saliva, starting at pH 8.0 with 112 millimols "total CO_2" (in contact with 6 percent carbon dioxide in air, column 5, table 1), if brought to the rumen at pH 5.7 (and in contact with 70 percent carbon dioxide in the gas), would therefore give off 89 millimols (112 minus 23) of carbon dixoide gas.

Under these conditions the 60 liters of saliva secreted daily by a cow would thus contribute as much as 119 liters of carbon dioxide to the rumen gas ($60 \times 0.089 \times 22$). This contribution would amount to about one half the daily rate of methane formation.

The effect of diffusion on methane and carbon dioxide content in the rumen will be discussed later.

Sources of Free Hydrogen in Rumen Gas

The traditional source of hydrogen in bacterial fermentations, according to Woods and Clifton (217), is carbohydrate. Kluyver (97), demonstrating his theory that hydrogen transfer is of primary importance in these reactions, describes a great variety of fermentations and shows that, in a considerable number of them, this transfer leads to hydrogen gas.

Hydrogen gas formation from cellulose has been observed by Popoff (151) in fermenting sewer mud and by Tappeiner (188) in rumen content as well as in hay fermenting *in vitro*. Neuberg and Arinstein (134) noted hydrogen gas formation during the fermentation of sugar to butyric acid or butyl alcohol. Letting rumen contents ferment *in vitro*, Markoff (111) observed that a high rate of hydrogen-gas formation was correlated with the abundance of soluble carbohydrates, particularly glucose or sucrose, in the fermenting medium. Addition of molasses to a ration of hay and crushed rye increased a hundredfold the ratio of hydrogen to methane in the gas developed. *In vivo* similarly, with a goat, Markoff noted 0.05 percent free hydrogen in the rumen gas when hay was fed, but 0.53 percent when beets were added to the hay ration.

Pyruvic acid can be a source of hydrogen gas, according to an investigation by Woodman and Evans (215) describing a fermentation process that yields acetic acid and equimolecular amounts of hydrogen and carbon dioxide.

In their study on the metabolism of *Clostridium tetanomorphum*, Woods and Clifton (217) have shown that not only pyruvate, fumarate, malate, glucose and maltose, but also a considerable number of amino acids may be sources for bacterial hydrogen gas formation.

The absence of free hydrogen in a gas mixture above a fermenting substrate does not necessarily indicate that no hydrogen gas has been produced, because some microorganisms consume hydrogen. Barker (23) carried out manometric measurements with *Methanobacter omelianskii* in an ethanol-bicarbonate medium. He noted rates of hydrogen consumption as high as 7 cc. hydrogen per hour per milligram of cell nitrogen—a rate just about 100 times that of oxygen consumption per milligram cell nitrogen of animal tissues, especially rat embryos (95).

Possibly, therefore, hydrogen in the rumen may in some cases be consumed as fast as it is produced; and this possibility, along with the others mentioned above, may explain discrepancies in results on ruminal hydrogen-gas formation.

Sources of Hydrogen Sulfide in the Rumen

The most likely sources for the hydrogen sulfide in the rumen are sulfur-containing organic compounds, particularly amino acids. Hydrogen sulfide production has been studied during the decomposition of cystine and cysteine (Wohlgemuth, 1904; Sasaki and Otsuko, 1912; cited by Stephenson, 183)

Dougherty (45), analyzing rumen gas for hydrogen sulfide by the method of Christensen and Wong (31), found concentrations as high as 0.7 percent hydrogen sulfide in rumen gas from steers that had died from bloat 3 to 12 hours before the samples were taken.

Olson (142) reported that in bloated animals the hydrogen sulfide concentration in the rumen may be ten to twenty times that in animals on dry feed—a statement suggesting that bloat-producing feeds contain a particularly efficient source for hydrogen sulfide. Kleiber, Cole and Mead (94), on the other hand, measuring the hydrogen sulfide concentration at the moment the gas leaves the rumen, found no relation between hydrogen sulfide concentration in rumen gas and severity of bloat. This problem calls for further research.

Sources of Oxygen and Nitrogen in Rumen Gas

The development of free nitrogen by bacterial action, though a theoretical possibility, is unlikely to be important in the rumen. Tappeiner (188) never found nitrogen in gases evolved from rumen content *in vitro*.

Air swallowed may be one of the sources of free nitrogen and oxygen often found in rumen gas. This process may reach considerable proportions: Quin (157) speaks of the establishment of an aerobic state in the rumen associated with swallowing air. If swallowing air were the only source of free nitrogen and oxygen in rumen gas and belching the only way to get rid of those gases, then their molecular ratio in rumen gas should be 4 nitrogen to 1 oxygen as in air. In the analyses of Kleiber, Cole and Mead (94) this ratio is mostly considerably greater. It averages 7 nitrogen to less than 1 oxygen. The widening of this ratio indicates that processes other than swallowing and belching control the volumes of at least one of these gases in the rumen. Absorption of oxygen in aerobic reactions in the rumen is probably the major factor. The effect of diffusion to and from the blood on the nitrogen to oxygen ratio in rumen gas will be discussed later.

Gas Diffusion between Rumen and Blood

Since it is, at least theoretically, possible that all the oxygen and nitrogen found in the rumen may have entered it by diffusion from the surrounding tissues and the blood, no discussion on the sources of rumen gases would be complete without taking into account the diffusion between rumen and blood.

a. Gas diffusion into or from internal cavities in general. Tobiesen (193) observed that the gas in a pneumothorax gradually reached a uniform composition of 90 percent nitrogen, 4 percent oxygen, and 6 percent carbon dioxide no matter which of these three gases had been used for establishing the pneumothorax. This composition was so constant that a change, namely the absence of oxygen, could be used as a valuable early symptom for diagnosis of developing pleuritis. The constancy of pneumothorax gas may be explained as a state of diffusion equilibrium between the artificial cavity and the blood.

A similar observation for a natural cavity was made by Ylppö (221), who pumped 700 to 1,100 cc. of air, carbon dioxide, or oxygen into his own stomach and noted that after some time equilibrium was established between the residual gas in the stomach and the gases in the blood. The time required to reach this equilibrium could be used as an index for relative diffusion rates. Carbon dioxide diffused much faster than oxygen, and nitrogen had the lowest diffusion rate.

Haggard and Henderson (67) investigated gas diffusion in the animal body further by injecting 1 liter of air, or nitrogen, or oxygen, or a gas mixture rich in carbon dioxide, into the peritoneal cavity of a dog. Samples of the intraperitoneal gas were analyzed at intervals and compared with gas analyses of blood samples taken simultaneously. When either nitrogen or oxygen was injected intraperitoneally, equilibrium of the carbon dioxide tension with the partial carbon dioxide pressure in alveolar air was established within 1 hour. Oxygen partly consumed by the peritoneum diffused much more slowly than carbon dioxide, and equilibrium of oxygen tension with venous blood was established in time.

Teschendorf (192), in a similar study, injected 100 cc. of various gases and vapors into the peritoneal cavity of rabbits and measured the time of disappearance of this gas as indicated by the x-ray picture. For gases with slow diffusion rates this method may be criticized because the total volume of gas in the cavity, as indicated by the x-ray picture, may be affected by gases diffusing into the cavity from the blood.

The resorption time for the 100 cc. of gas increased from 4 minutes with hydrogen sulfide to 1 hour with carbon dioxide, 17 hours with carbon monoxide, 1 day with hydrogen, methane, or oxygen, and 3 to 4 days with nitrogen. For most gases the absorption time followed Exner's law:

$$\frac{1}{t} = k\frac{\alpha}{\sqrt{\delta}}$$

where t = absorption time, α = absorption coefficient for the gas in water (Bunsen coefficient), δ = relative density of gas compared to air = 1, and k = a constant.

According to Teschendorf, oxygen and carbon monoxide absorbed faster than Exner's law would predict because they react with hemoglobin. This explanation is not convincing: the reaction with hemoglobin would only tend to keep the diffusion potential constant, which condition for other gases is accomplished by other processes such as exchange with air in the alveoli of the lung. Reaction with the peritoneal epithelium itself would seem to be a better explanation for the relatively fast resorption rate of oxygen. If one considers the differences in diffusion potential involved, especially

comparing nitrogen with methane or hydrogen, the agreement of Teschendorf's results with Exner's law seems surprising.

The form in which carbon dioxide diffuses through living tissue is of particular interest. Osterhout and Dorcas (145) demonstrated that carbon dioxide enters normal algae (Valonia) only as an undissociated molecule. A similar observation was reported for animal tissue by Wehrli-Hegner and Wyss (205), who measured penetration rates through venal membranes of frogs.

b. Permeability of rumen wall. Since the rumen wall, unlike the membranes in the peritoneal cavity or the pleural membrane, is lined with several layers of cornified cells, the question is justified whether the results on diffusion of gases in the animal body, discussed above, are applicable to gas exchange through the rumen wall or whether this wall is a barrier to such exchange.

Several observations on record indicate that the rumen wall is indeed permeable to diffusable substances. By freezing-point measurements Davey (39) demonstrated that the osmotic pressure in rumen liquid is equal to that in blood. If this observation should be generally confirmed, the most reasonable explanation would be a diffusion equilibrium between rumen and blood, which is likely only when the rumen wall is permeable. Such permeability was demonstrated by Rankin (161), who discovered that glucose is absorbed through the rumen wall. McAnally and Phillipson (117) and Barcroft, McAnally, and Phillipson (18) determined the concentration of volatile acids in the veins draining the rumen of sheep. They noted a two- to tenfold increase in this concentration over that in jugular blood when the sheep had been fed. Acetic acid was prominent among these acids thus determined. No volatile acids were found in blood from starving sheep. Acetate added to the rumen content through a fistula led to an immediate rise in the volatile acid content of the blood. Propionate produced a smaller increase, indicating a slower diffusion; and butyrate had the lowest rate of transfer among the three. In lambs, 2 to 4 grams of acetic acid per hour was transferred from rumen to blood stream.

c. Gas diffusion through rumen wall. Definite and reliable data for the diffusion rate of gases through the rumen wall are still lacking. That this diffusion plays an important role for the composition of rumen gas has been realized by Zuntz and his co-workers (222). They noted that gas evolved from rumen content *in vitro* contained 3 to 7 volume parts of carbon dioxide for one of methane, whereas gas samples taken directly from the rumen of goats several hours after feeding had about the same percentage of carbon dioxide as of methane. This comparison led the same investigators to conclude that by far the greatest part of the carbon dioxide developed in the rumen is transferred to the blood.

A rough idea of the extent of carbon dioxide diffusion from the rumen may be gained by using Teschendorf's (192) observation that 100 cc. of carbon dioxide was resorbed through the peritoneum of a rabbit in 1 hour. The daily rate in this case would thus amount to 2.4 liters of carbon dioxide. The diffusion potential (difference in partial pressure) may be estimated as 0.94 atmosphere. If the diffusion area of the rumen of a cow is 50 times as great as that which was effective in the rabbit, and if the carbon dioxide diffusion potential is 0.64 atmosphere, and if, furthermore, the diffusivity of carbon dioxide through the rumen wall is equal to that through the peritoneum of the rabbit, then the daily rate of carbon dioxide transfer from rumen to blood would be 82 liters ($2.4 \frac{0.64}{0.94} 50$). That would be 3.4 liters per hour.

A cow studied by Cole, Mead and Kleiber (35) gave off (through a rumen fistula) rumen gas at a rate ranging from 11 to 20 liters per hour for the first 5-hour period after feeding. Assume that this gas contained 65 percent carbon dioxide, the mean of 12 analyses by Washburn and Brody (204), for the first 5 hours after feeding. Then the cow lost through the fistula from 7 to 13 liters of carbon dioxide per hour. The diffusion rate of carbon dioxide estimated above would thus range from 25 to 47 percent of the carbon dioxide given off through the rumen fistula. This is a somewhat smaller part played by diffusion than Zuntz and his co-workers assigned to it.

d. Blood as source for nitrogen and oxygen gas in the rumen. Washburn and Brody (204) explain why, in their opinion, it is unlikely that increases of oxygen and nitrogen percentages in rumen gas are due only to diffusion of these gases into the rumen. Since oxygen diffuses about three times as fast as nitrogen (in Teschendorf's trials), Washburn and Brody conclude: "If diffusion were the only process taking place along with increasing percentages of oxygen and nitrogen, the percentage of oxygen should be about three times that of nitrogen." This argumentation neglects the fact that diffusion rates depend not only on diffusivity but also on diffusion potentials.

The order of magnitude of nitrogen diffusion into the rumen may be roughly estimated by a procedure similar to that used above for carbon dioxide diffusion.

According to Teschendorf's (192) measurements, 100 cc. of nitrogen disappeared from the peritoneal cavity of a rabbit in 3 to 4 days. The rate of transfer thus was about 30 cc. per day. The effective diffusion potential in this case may be estimated to 0.2 atmosphere, since pure nitrogen was injected into the peritoneal cavity and the blood is in equilibrium with air containing 80 percent nitrogen. The potential for diffusion of nitrogen from blood to rumen gas containing 7 percent nitrogen amounts to 0.73 atmos-

phere (0.80 minus 0.07). If the rumen of the cow has a diffusion area 50 times as great as that effective in Teschendorf's rabbit and if the diffusivity in both cases is the same, then the daily rate of nitrogen diffusion into the rumen amounts to $0.3\frac{0.73}{0.20}$ 50, or 5.5 liters.

Gas diffusion between rumen and blood requires and deserves a great deal more research with methods available since the invention of the cyclotron, the Geiger counter, and the mass-spectrometer.

Factors Influencing Rate and Type of Gas Formation[9]

The gases formed in the rumen and their sources have been considered. Next let us discuss means of influencing the rate of formation of all or of specific gases within the rumen.

Type of Feed

A view held until recently was that only under conditions in which bloat was common was there an appreciable formation of gas in the rumen. From evidence now at hand, however, the feeding of bloat-provoking diets does not necessarily result in greater gas formation than from those not usually associated with bloat (35, 204, 84). Quin (156) likewise reports that the amount of gas produced on alfalfa hay is comparable with that on green alfalfa. It is well known that bloat is frequent on green alfalfa, rare on alfalfa hay. His finding that no gas is produced on a diet of alfalfa hay and maize is opposed to the results of other investigators using similar diets in which other grains have been substituted for maize. This finding, however, may depend upon the time of feeding the alfalfa in relation to the time when gas formation was studied. He reports several observations that warrant further attention. For example, on a basal diet of alfalfa hay, the feeding of glucose initiated a rapid formation of gas; but if the basal diet consisted of poor grass hay, the feeding of sugar resulted in little or no gas production. The rapid production of gas following ingestion of sugar and the much slower formation following ingestion of starch are also of interest. The influence of prior feeding on gas production which Quin describes is possibly a clue to some of the hitherto mysterious outbreaks of acute tympany.

McAnally (116) also showed that sugar added to rumen ingesta stimulated gas production; but Jacobson, Espe, and Cannon (84) found that sugar tended to inhibit it. These latter workers do not give the exact amounts used.

According to Jacobson, Espe, and Cannon (84), the addition of silage to an alfalfa-hay and grain ration depresses gas formation. The results would be more impressive, however, if these writers had specified the total digest-

[9] This section prepared by the late T. M. Olson.

ible nutrients ingested under the two feeding regimes, in order that the effect of silage could be clearly differentiated from the effect of total food consumed. At any rate, the clear-cut differences which they observed warrant studies on preventing bloat on legume pastures by supplemental feeding of silage.

The studies referred to above are concerned with the effect of feed upon total gas production. Another series of investigations has centered around the effect of feed upon specific ruminal gases. Dougherty (43) reported 0.2 percent carbon monoxide in ruminal gas when hay was fed, 0.2 to 0.3 percent when freshly cut clover was given. Carbon monoxide insufflated into the rumen was absorbed and had a marked physiological effect. In later studies (44), however, the amounts of carbon monoxide produced on dry and green feeds appeared to be comparable; but greater amounts of another toxic ruminal gas, hydrogen sulfide, were apparently produced upon green than upon dry feed. *In vitro* and *in vivo* experiments indicated that the addition of sulfur, cystine, or methionine to ruminal ingesta increased hydrogen sulfide production. Comparatively large amounts of hydrogen sulfide in ruminal gas of animals presumably killed by bloat were reported (44, 45). Since the samples were taken for analysis several hours after death, it is impossible to state what the concentration of hydrogen sulfide was prior to or at the time of death. Incidentally, three samples were obtained from bloated animals on dry feed, and one sample from an animal on Ladino clover pasture.

Olson (142, 143) also reports higher hydrogen sulfide values for ruminal gas from bloated than from normal animals, and he likewise found higher hydrogen sulfide values from cows on green legumes than from those on dry feed. These samples were taken from dead animals.

In a later study using a different method for determining hydrogen sulfide values, Olson and Breazeale (unpublished) found values ranging from 0.0418 to 0.17, with an average of 0.068 percent for cows 1 to 4 hours after pasturing on alfalfa as compared with an average of 0.0038 percent before feeding; that is, the feeding caused an increase of approximately 18 times.

Alway (5) reported wide variations in sulfur content of legumes, dependent upon the sulfur content of the soil. According to Painter (146), the protein of legumes is higher in sulfur than the protein of nonlegumes. Analyses also indicated that the leaves of legumes contained more than twice as much sulfur as the stems of the same plants.

Kleiber, Cole and Mead (94) found no difference, however, in the percentage of hydrogen sulfide in ruminal gas of animals on diets that produced bloat as compared with diets on which bloat did not occur. The cows were on alfalfa pasture in both instances; but the feeding of sufficient Sudan hay to prevent bloat did not result in a decline in hydrogen sulfide, as one would expect if it were the factor responsible for bloat. Furthermore, the hydrogen

sulfide values for living bloated animals were comparable with those of normal animals.

Amount of Feed Consumed

Studies by several investigators (204, 35, 156) indicate that the rate of gas production in the rumen is roughly proportional to the amount of feed consumed. For example, 11 liters of gas were produced over a 5-hour period after consumption of 4 pounds of alfalfa hay, as compared with an average of 77 liters for two trials on 25 pounds of alfalfa (35). This explains why greedy feeders are more subject to bloat than poor feeders. If, on the other hand, a greedy feeder has an unusual capacity for expelling ruminal gas, she may still not be susceptible to bloat.

Olson and Breazeale (unpublished) found the hydrogen sulfide percentage to be greatest in the animals that ate most.

Time of Previous Feeding

Washburn and Brody (204) were the first to show that eating stimulated gas production and that the peak in the rate of gas production came soon after feeding, followed by a gradual descent in which very little gas was being formed 24 hours later. All other investigators (35, 84, 156) have confirmed this general statement. Johnson and his co-workers (85) found a similar relation between feeding and the formation of methane. Although Quin (156) agreed, in general, with the others, he noted that after ingestion of certain feeds—for example, maize and alfalfa hay—no gas was produced in certain instances. His results, however, were obtained with sheep, whereas all other investigators used cattle. Olson and Breazeale (unpublished) found the greatest increase of hydrogen sulfide about 2 hours after the cows began eating.

This relation between eating and gas production is of great practical importance. It affords an explanation as to why cows usually bloat while on alfalfa pasture or shortly thereafter. In the experience of Cole and his coworkers (unpublished), rarely does bloat develop later than 1 hour after removal of cows from alfalfa pasture. These authors have noted that the feeding of grain immediately after cows are taken from alfalfa pasture may induce or accentuate bloat; yet occasionally the act of eating grain seems to facilitate belching in mildly bloated animals. From these experiences one can conclude that animals not bloated 1 hour after removal from pasture can safely be left without further attention.

Type of Microorganisms

Some have postulated that the type of microorganisms present in the rumen has an influence on gas formation. Koffman (100) concluded that the

number of small protozoa was increased in bloated animals, whereas the reverse was true of the large protozoa. Quin (156, 157) believes that pseudo-yeast cells on alfalfa plants may result in an excessive gas formation after ingestion of this legume. Others (35, 84, 204), however, have not confirmed his finding that the consumption of green alfalfa leads to excessive gas formation.

Johnson and his collaborators (85) give interesting information on the relation of microflora in the rumen to time after feeding. At 16 hours after feeding, the bacteria were reduced to one-thirteenth the number found at the end of the first hour after feeding. Yeastlike organisms followed a similar but less clear-cut trend, while a reciprocal relationship existed regarding the protozoa. After two days of fast, however, rumen contents were practically free of protozoa, a finding in accord with those of Ferber (53).

Even though the main problem in bloat seems to center around the expulsion of gas rather than its formation, further study must be given to this whole problem of the role played by microorganisms in gas production and the effect of different feeding regimes upon the microflora of the rumen.

Influence of Other Factors on Gas Production

Jacobsen, Espe and Cannon (84) studied the influence of various factors on the rate of fermentation of ruminal ingesta *in vitro*. They drew the following conclusions:

> Changes in dilution, temperature and hydrogen-ion concentration, in the ranges normally occurring in the stomach, had little effect on the rate of fermentation. Amounts of salt which the cow would tolerate in her drinking water also had little effect in *in vitro* studies. Common minerals, such as limestone, accelerated fermentation probably through neutralization of the acids formed. Of a number of other materials tried, acetic and lactic acids tended to depress fermentation most severely while sodium formate had the greatest accelerating influence.

Myburgh and Quin (133) studied the effect of adding acid and alkali to ruminal fluid *in vitro* upon gas formation. Not until sufficient acid had been added to reduce the pH to 6.5 at the end of 20 minutes was there a perceptible depressing effect upon the amount of gas evolved. When sufficient alkali had been added to increase the pH at the end of 20 minutes to 8.35, the rate of fermentation was reduced to about one third the rate of untreated ingesta.

Another factor that may markedly affect the rate of gas production is the rate of passage of material through the rumen. Mead and Goss (127) encountered severe bloat in cattle restricted to a grain diet for long periods. Cole and Mead (unpublished data) studied the incidence of bloat on this same diet. Over a 7-week period only one mild case of bloat occurred even though the cows consumed 40 pounds of grain on certain days—a level of feeding exceeding that employed by Mead and Goss. One interpretation of

this difference dependent upon length of time on the diet, is that over a long period of time atony of the rumen develops; food then remains in the rumen longer, and the result is greater gas formation. With an absence of coarse food in the diet, one would expect a faulty eructation mechanism, which would explain the bloat that occurred after long periods on grain alone. Mead and others (125) found that, although bloat was produced on finely ground hay and grain, it was not severe. Possibly here, again, this finely ground material moved from the rumen too rapidly for severe bloat to develop. Other theories could just as well be proposed to clarify these findings —for example, changes in the microflora of the rumen may afford an explanation. Further studies on the rate of gas production and the rate of passage of these foods through the rumen are essential.

Swallowing of air should perhaps also be mentioned, not as a factor influencing gas production, but as a factor influencing the total amount of ruminal gas. Gastric tympany resulting from swallowing of air is not uncommon in man (6); but, judging from the small amounts of nitrogen in ruminal gas (204, 35, 94) of both normal and bloated animals, it is not an important factor in ruminants. Because, however, some livestock producers still maintain that bloat is caused by "swallowing of wind," this view seems worthy of mention.

Expulsion of Gas from the Rumen[10]

The dorsal portion of the rumen cavity constitutes the gas-storage space of the ruminant stomach. This space varies in capacity according to the degree of filling of the rumen with liquid and solid ingesta and according to the tone of the ruminal musculature. A noteworthy anatomical fact is that the esophagus communicates with the rumen cavity at a much lower level than the upper limits of the dorsal rumen sac. This structural arrangement would appear to offer considerable interference with the freedom of movement of gas to the esophagus.

It is now generally agreed that gas is expelled from the rumen, for the most part, by eructation. Wild (210), studying the frequency of eructation under a variety of conditions in cattle, sheep, and goats, concluded that eructation is a normal and absolutely necessary process in ruminants. He observed that increased abdominal pressure as in coughing, defecation, or urination facilitated belching. Coughing is without doubt an important accessory process brought into play to eliminate ruminal gas in cases of mild bloat. Cole and his co-workers (unpublished data) have observed frequent coughing in both bloated cows and sheep, though it would appear to be brought into play more frequently in the latter. Turpentine administered to

[10] This section prepared by H. H. Cole. Many suggestions were contributed by R. S. Amadon.

bloated cows often results in coughing, with consequent elimination of ruminal gas.

The exact amounts of ruminal gas that pass to the blood stream and are exhaled are not known. (See discussion under "Sources of Rumen Gases.") It is known, however, that diffusion from the rumen is inadequate to prevent death from bloat when the esophagus is no longer an avenue of escape.

Role of the Cardiac Sphincter in Eructation

Aggazzotti (2) concluded that the cardiac sphincter is normally relaxed. This conclusion was reached because, when a sheep was placed on its back with its head downward, some ruminal contents leaked into its mouth at each inspiration. Despite this evidence, obtained under highly artificial conditions, it seems certain that the cardiac orifice is normally closed and, furthermore, that the orifice is opened reflexly in eructation, not simply forced open by increased ruminal pressure. The fact that intra-ruminal pressure is subatmospheric when the rumen is relaxed (182, 208, 158, 35) shows clearly that the orifice is not normally open. Stigler (186) who palpated the cardiac orifice through a rumen fistula, found that it was normally closed. The cardiac orifice is not opened simply by increasing ruminal pressure; this fact has been demonstrated by elevating the ruminal pressure artificially by introducing gas into the rumen and noting that belching occurs only when the rumen is in an active state of contraction (35).

Amadon and Detweiler (personal communication) palpated the cardia at the moment of eructation by inserting the hand through a rather small rumen fistula, the opening of which was sealed off with the inserted arm. At the moment of eructation they found the cardia widely dilated, assuming a funnel-like appearance. This action, they concluded, involved muscular contraction and is therefore an active rather than a passive change. They noted, however, that at times the cardia may not actively dilate, but relaxation occurs so that gas may escape through the orifice.

Wester (208) observed that belching could occur in an animal with an open-rumen fistula, a fact indicating that increased ruminal pressure is not essential. Thus it seems certain that opening of the cardiac orifice for the expulsion of gas is a reflex coordinated with ruminal contraction. Hoflund (77) and Wester (208) likewise observed that eructation is associated with ruminal contraction, but not with every ruminal contraction. In the opinion of the latter, the opening of the esophagus depends upon the strength of the ruminal contraction, more particularly upon the contraction of the transverse folds.

Although in eructation the cardiac orifice is opened during active contraction of the rumen, in regurgitation it is opened with the rumen quiescent

(170). In swallowing, on the other hand, the opening of the orifice bears no relation to the phase of activity of the rumen.

Jacobsen, Espe and Cannon (84) visualized that bloat is due to a mechanical blocking of the cardiac orifice with rapidly ingested food. In other words, according to this view, legumes are ingested more rapidly than they can be stowed away in the posterior rumen. In support of their hypothesis, these writers give evidence that alfalfa is consumed more rapidly than blue grass, thus explaining why bloat does not occur on the latter. This explanation for the lack of bloat on grasses is untenable, however, in the light of experiments by Cole and Kleiber (unpublished data). The latter workers found that one cow, under observation to determine the relation between feed consumption and bloat, bloated regularly upon consuming as little as 12 to 18 pounds of alfalfa pasture. This same cow ate 47 pounds of Sudan pasture over a similar period without bloating. It is difficult to reconcile these findings with the "piling up" theory of Jacobsen and his co-workers. Another interesting observation was made on this animal. When she was given 7 pounds of Sudan hay just before pasturing on alfalfa, she still continued to bloat. At certain times when she was bloated, audible eructations occurred at the rate of five to seven per 5-minute intervals but still she remained bloated for an hour or more. It seemed, therefore, that this cow had a partial obstruction of the esophagus; but the later finding of complete lack of bloat on Sudan pasture did not support this view. Rather it would appear, that alfalfa furnished insufficient stimulus to elicit a normal and complete eructation. Data on gas formation under the two circumstances, however, are lacking. One may inquire why the Sudan hay fed before pasturing on alfalfa did not provide sufficient stimulus. As will be pointed out in a later section, this would appear to be a quantitative problem.

Amadon and Detweiler (personal communication) performed an experiment relevant to the mechanical obstruction theory of Jacobsen and his coworkers. The rumen was filled excessively by transferring rumen ingesta from one rumen-fistula cow to another and then sealing off the fistula of the excessively filled rumen with a pneumatic plug. Belching was not prevented by this procedure.

Greeberg, Visscher, Petersen and Boyd (64) did not observe mechanical obstruction in goats turned on their backs. Two goats died within 5 hours in this position, and the third in 2 hours. Death was caused by pulmonary congestion, alveolar hemorrhage and interstitial edema. Eructation was normal, and there was no evidence of tympany. When and how much the animals were fed before the experiment is not reported. If they were not given an abundance of feed immediately preceding, this experiment does not rule out bloat as a possible contributing factor in the death of animals turned on their backs.

According to another view, not involving dysfunction of the cardiac sphincter in a certain type of bloat, the gas becomes so intimately mixed with the food mass in the rumen that it cannot escape. Wester (208) and McIntosh (122) subscribed to this view; and the theory has been recently amplified by Quin (157), who attributes "foamy bloat" to a high saponin content of the ingested plants. This theory will be discussed in greater detail in another section.

Further evidence that the cardiac orifice is opened reflexly during eructation is obtained by severing the vagi. Under these circumstances there is a lack of normal eructation, and tympany results (51, 110). This fact implicates the nervous system with the act of eructation, but, admittedly, failure of eructation may be due to ruminal atony.

Role of Rumino-reticular Activity in Eructation[11]

As stated above, eructation is synchronized with ruminal contraction, and there is good reason to believe that eructation rarely occurs in cases of ruminal atony. According to Amadon (personal communication), ruminal contraction is essential in eructation in order to move the air pocket anteriorly to the cardiac orifice. Figures shown by Magee (106) also indicate that this is the case. Furthermore, Amadon reports that the dilation of the reticulum which accompanies ruminal contraction lowers the liquid level in the anterior rumen so that the orifice is unobstructed. Perhaps a sudden spasm, as in coughing, involving sudden pressure changes, may result in a forward movement of the air pocket without ruminal contraction.

Eructation does not occur at every ruminal contraction. Wester (208) believes, as previously mentioned, that the opening of the esophagus is associated with an extra strong contraction of the transverse folds. If this is true it is not reflected in an unusual increase in ruminal pressure (Cole, Mead and Kleiber, 35).

In bloat, is there a lack of ruminal contractions or simply a lack of opening of the cardiac orifice? The answer would seem to depend upon the cause of bloat. For example, according to Begg (25), in bloat following peritonitis

[11] It is outside the province of the present paper to discuss articles on ruminal activity which have no bearing on eructation. Since ruminal motility is a necessary essential for eructation, however, some of the more important papers on ruminal motility should be cited: Schalk and Amadon (170), Phillipson (149), and Wester (208), recorded ruminal contractions by inserting a balloon connected to a manometer through a fistula into various compartments of the rumen. Krzywanek and Quast (102) and Quast (154), used a modification of this method. Stålfors (182), Bergman and Dukes (27), Quin, van der Wath and Myburgh (159), and Cole, Mead and Kleiber (35) recorded activity of the rumen by recording the changes in ruminal pressure. Mangold and Klein (110), Marschall (114) and Aggazzotti (2) observed ruminal contractions in laparotomized animals. Czepa and Stigler (38), Magee (106) and Trautmann (197) studied ruminal contractions by means of x-ray. The author last named also studied movements of the rumen visually by making a window in the skin of the left flank. Electrical changes in the ruminal musculature accompanying ruminal movements have been recorded by van der Heyde (75) and Toman (195). Numerous other papers on ruminal motility or related subjects may be of interest (29, 49, 57, 58, 71, 88, 99, 103, 108, 152, 165, 169, 173, 179, 194, 196, 198, 199, 200, 209).

there is ruminal atony. Begg states further that, following acute tympany, atony of the walls of the rumen may result in chronic tympany. Cole and Kleiber (unpublished data) have evidence that ruminal atony is not involved in the first stages of bloat on green legumes. Using a tympanometer devised by Kleiber (unpublished) for the indirect determination of ruminal pressure, they find periodic changes in pressure due to ruminal movements in animals mildly bloated. After bloat becomes severe, however, ruminal atony may develop, resulting in little change in ruminal pressure. Wester (208) states that, rather than ruminal atony, there is an increase in ruminal contractions in the early phases of acute bloat. This is in accord with a finding of Amadon and Detweiler (personal communication)—namely, that increased intraruminal pressure causes a greater frequency of rumino-reticular contraction cycles and a greater strength of contraction. They conclude that this reaction to the distending affect of accumulated gas is very important, since it renders the muscular actions more effective in the process of gas removal. It is undoubtedly an important factor in spontaneous recovery from mild bloat. It would appear, therefore, that the primary failure in acute bloat results from the lack of opening of the cardiac orifice, not from ruminal atony. The information at hand is far from complete, however, and more detailed studies should be made.

Many types of chronic bloat, on the other hand, are due to ruminal atony. Bloat following peritonitis has been mentioned as an instance. Bloat accompanying digestive disturbances in calves probably result in part from ruminal atony. Possibly bloat resulting from the feeding of concentrates alone over long periods has a similar cause (Mead, Cole and Regan, 125). Conceivably ruminal atony associated with indigestion is the result of disturbances in more distal parts of the digestive tract. Phillipson (149) has shown that distention of the abomasum affects the contraction of both the rumen and reticulum. Bloat accompanying impaction is probably also due to a lack of normal muscular contractions. The possibility that toxic substances formed within the rumen itself affect ruminal motility must be considered.

Experimental Production of Bloat[12]

In order to attack intelligently the problems of preventing and treating bloat, one must understand the etiology of bloat. Until very recently, there have been no reliable means of producing bloat by feeding procedures; and this lack has proved to be the greatest handicap in the study of prevention and treatment. As late as 1942, Dougherty (45) and Jacobsen, Espe and Cannon (84) report their inability to produce bloat at will on legume pastures. Several methods are now available, however, for producing bloat; and

[12] This section prepared by H. H. Cole.

these should be a great impetus to studies relating to cause, prevention, and treatment.

Section of the Vagi

The work of Ellenberger in 1883 (51) demonstrated that bloat could be produced by section of the vagi. Although this is a drastic procedure, it is surprising that this knowledge has not been employed in studying this pathological condition. Perhaps refinement of the method to the extent of merely cutting the branches of the vagi supplying the lower esophagus and the rumen, thus not interfering so greatly with the general welfare of the animal, would make this a more practical tool in bloat studies.

Use of Drugs

According to Dougherty (personal communication), a conspicuous feature of poisoning with phosgene gas in goats is the development of bloat. Histamine paralyzes the rumen musculature (45). Petersen (personal communication) found that the intravenous injection of histamine produces marked bloat; and also reports bloat with what is apparently an anaphylactic shock. In an attempt to study the body deposition of fat, homogenized Sudan III stained corn oil was administered, intravenously, to an 8-month-old Holstein on a normal ration of grain and alfalfa hay. No symptoms followed the first injection. During the second injection, made 12 days after the first, the animal collapsed; and such extensive tympany followed to necessitate rumen puncture. Eight subsequent injections were followed by diminishing severity of symptoms. Bloat, however, occurred each time. A more detailed study of how these drugs produce bloat may help in building up our knowledge of the condition. Wynn-Lloyd (218) reports that the intra-tracheal injection of chloroform produces tympany in a large proportion of cases, if the animals have been fed before the injections.

The methods considered up to this point for producing bloat are artificial means. Nevertheless, they may be very important for solving the bloat problem. We now wish to consider, however, the production of bloat by feeding procedures.

Green Legumes

First we shall consider the production of bloat with green legumes. Acute tympany did not become a serious problem, according to Niborg (135), until in the middle of the eighteenth century, the pasturing of clover became a common practice. Although bloat has occurred in ruminants pastured on legumes in many parts of the world, not until 1943 did Cole, Mead and Regan (36) report that the condition could be produced on alfalfa pasture

any season of the year, and under varying environmental conditions, if the alfalfa was in an appropriate stage of development. Results obtained in the 1942 season have been repeatedly confirmed in 1943 (Mead, Cole and Regan, 125) and 1944 (Cole and Kleiber, unpublished data). It is unfortunate, however, that studies have been limited to one section of the country, with alfalfa under irrigation.

The following is the list of essentials which these workers considered important if bloat is to be produced on alfalfa pasture: 1. The alfalfa should be in a crisp, succulent stage and should be thick enough to allow for rapid ingestion. The studies indicate that very young alfalfa is not very palatable when pastured without previous feeding of hay, and thus the alfalfa should be pastured during the latter part of the crisp stage. Since alfalfa usually becomes wiry during the bloom stage, it should be pastured before bloom. 2. The animals should be deprived of all hay and straw, although it is reported (36) that fine-stemmed alfalfa did not completely prevent bloat under the experimental conditions and that 5 pounds of straw (125) was not satisfactory as a preventive. 3. The feeding of grain does not appear to be very significant—bloat was produced in animals which did, and did not, receive it (125). The feeding of grain just before pasturing shortened the interval between the beginning of pasturing and the appearance of bloat; but, on the other hand, this appeared to lessen the severity of bloat, because the animals bloated before much alfalfa had been consumed. 4. The alfalfa pasture should be free of grasses and weeds. Cattle seem to prefer these plants to the succulent alfalfa. The result is probably unfavorable for the production of bloat for two reasons: the amount of feed consumed is decreased, and the grasses and weeds have some preventive action. 5. There should not be any appreciable amount of old, long, tough stems of alfalfa left from the previous pasturing period. If necessary, the field should be clipped before the growth of alfalfa to be used appears. 6. One should provide the alfalfa with sufficient water to induce rapid growth. If this is not done, the alfalfa will not be succulent enough. In many areas, therefore, it will be necessary to irrigate rather than to depend upon rainfall. In the experiments of Cole and co-workers, there was no appreciable rainfall during most of the pasturing period, and thus all water was supplied by irrigation. 7. Severe bloat can be produced by feeding alfalfa tops in the barn (125). This procedure has an advantage in that feed consumption can be easily determined. Furthermore, fields can be used which otherwise would be unsatisfactory. For example, if an alfalfa field is contaminated with low-growing grasses, these grasses would be detrimental to bloat production by pasturing, but are avoided by cutting the alfalfa tops. Or it may be that a field is contaminated with weeds and grasses along the fence. It was found that green alfalfa could be stored

for 24 hours in small piles in a well-aerated barn. An unsuccessful attempt was made to produce bloat by the use of dried alfalfa tops. The failure of these dry tops to produce more than mild bloat is attributed to the more irritating effect of the dried material, but the point needs further investigation.

Grain Alone

Mead and Goss (127) reported bloat on a non-roughage diet for cattle. McCandlish (119) encountered bloat when milk was the sole diet for calves. Mead, Cole and Regan (125) interpreted these findings on the basis that such diets did not contain coarse irritating roughage; but a more recent finding of Cole and Mead (unpublished data) indicates that, although this may be one predisposing factor, another appears to be involved. These latter workers fed the same grain mix used by Mead and Goss over a 7-week period to lactating cows, and in that time only one mild case of bloat was encountered. If the difficulty were merely a lack of sufficient coarse roughage, bloat should appear within 48 hours if feed consumption were sufficiently high, judging from results on pasturing alfalfa. Apparently an additional factor, ruminal atony, for example, may have been involved in the longtime experiments of Mead and Goss.

Ground Hay and Grain

Mead, Cole and Regan (125) who compared the incidence of bloat on ground hay and grain with unground hay and grain, found 21 mild cases on the former and only one on the latter ration. Since the same hay was used in the same proportion to grain, this experiment gives conclusive proof that the physical condition of the feed is an etiological factor in bloat.

Severe bloat was not produced on the ground hay and grain ration. More information is needed before the lack of severe bloat can be explained. Conceivably, the feed when finely ground may pass through the rumen too rapidly for severe bloat to develop, or perhaps the activity of the microorganisms is decreased. It is known that the animals consumed enough for bloat to occur, judging from the amounts necessary to produce bloat on green alfalfa. Determination of the rate of gas formation and the rate of passage of food through the rumen would allow for a better-founded explanation.

Individual Susceptibility to Bloat

Some animals are much more susceptible to bloat than are others; and yet very few lactating dairy cows will be entirely free of bloat if kept on bloat-provoking pastures for any length of time (36, 125). There are, as yet, unexplained variations in individual susceptibility not dependent upon food intake (125). It is true, generally speaking, that the more greedy feeders are

more susceptible than the less greedy; and this fact doubtless explains the greater susceptibility of lactating as compared with dry cows (125).

On an occasion of feeding green alfalfa tops (125), one animal was bloated after consuming only 8 pounds of green alfalfa; but most of the others did not bloat until 60 to 90 pounds had been consumed. Judging from the data obtained by these authors on feed consumption while pasturing and the time elapsing between the beginning of pasturing and the onset of bloat, these figures on the amount consumed before bloating occurs are characteristic of pasturing conditions as well. Bloat occurred as early as 28 minutes and as late as 7 hours after the animals were turned into pasture.

Prevention of Bloat[13]

Studies on the prevention of bloat have been handicapped until recently by inability to produce bloat at will. This phase of the subject therefore requires further investigation. Since the feeding of roughage prior to pasturing has more experimental evidence than other preventive procedures, it will be discussed first.

Feeding Hay before Pasturing

The feeding of hay before pasturing has long been accepted as one of the procedures for preventing bloat on legume pasture, but some have reported that it is not effective (140).

Cole, Mead and Regan (36) put this preventive procedure to experimental test on fields which had been tested for their capacity to produce bloat. The overnight feeding of alfalfa hay did not always prevent bloat; coarse alfalfa was more effective than fine-stemmed leafy alfalfa. On the other hand, overnight feeding of Sudan hay was completely effective. More recent studies by Cole and Kleiber (unpublished data) have shown that 10 pounds of Sudan hay would not protect all cows, whereas 17 pounds did so. Mead, Cole and Regan (125) found that 5 pounds of barley straw was not sufficient. These authors suggest an explanation. When straw is fed, the animals consume more alfalfa than when no straw is given. This greater consumption increases gas production, and because of this the tendency for bloat is increased. At the same time, however, the straw consumed provides a greater stimulus for eructation, so that the end result is not greatly different than when the cows receive no straw.

From these studies it becomes obvious why hay has not always been found to prevent bloat. Sudan hay was the only roughage studied which was completely effective, and it had to be supplied overnight if the cows were to eat enough to be protected. When cows were fed only the 2 hours before

[13] This section prepared by H. H. Cole.

pasturing, they consumed only 14 pounds. This amount protected most, but not all. Consequently, a roughage of poor palatibility will not be effective because of the small amount taken. On the other hand, very leafy hays such as fine-stemmed alfalfa are not effective even though considerable is consumed.

It may appear to be a costly procedure to feed all the palatable hay that cows will eat overnight to prevent bloat on alfalfa pasture the following day. The studies of Cole and Kleiber (unpublished data) indicate, however, that the consumption of alfalfa pasture is reduced only about 25 percent by feeding hay overnight and, therefore, that this is a method for increasing the consumption of roughage, thereby decreasing the amount of grain required.

These studies were conducted on pastures producing maximum bloat and with cows known to be susceptible. For example, in the studies of Cole and Kleiber all eight cows on test bloated one day when Sudan hay was not fed, and some had to be treated. Under most conditions, smaller amounts of hay would probably provide protection.

Certainly with bloat, an "ounce of prevention is worth a pound of cure." Thus methods of prevention deserve much further study. Other roughages should be used. Attempts should be made to provide an index for the bloat-provoking capacity of different alfalfa pastures and an index for the protective values of different roughages. Because pastures and hays vary in so many respects, these indices could never be strictly quantitative; but at least they would serve as a rough gauge.

Alternating Sudan and Legume Pastures

Overnight pasturing on Sudan has proved effective in preventing bloat on alfalfa pasture the following day (36). Sudan has an advantage over most grasses in that it is very palatable and its nature of growth permits rapid ingestion. The scabrous character provided by its barbed leaves is also suggested as an advantage in inducing eructation.

The question arises as to whether the same effect could be produced by allowing cattle to use simultaneously adjoining Sudan and alfalfa pastures. In a practical test, Regan and Mead (unpublished data) turned heifers into a field where they had access to alfalfa on one end and Sudan on the other. This did not prove feasible, for they ate practically no alfalfa until the Sudan was completely eaten off.

Other Preventive Measures

One of the most feasible procedures is to mix grasses with legumes; but, unfortunately, no well-controlled experiments of the kind have been made. Practical experience has shown that if the grasses make up at least 50 per-

cent of the mixture there is no difficulty with bloat (26). One difficulty encountered is in maintaining a proper proportion at all seasons. This problem can probably be best solved by the joint efforts of animal husbandmen and agronomists.

Another practical procedure is to pasture legumes only after they reach the early bloom stage. Again, there have been no well-controlled experiments to determine the limitation of the procedure.

Continuous day and night pasturing is frequently suggested as a means of preventing bloat. The authors have seen this procedure fail even with dry cows. It is dangerous because the animals may bloat during the night and thus die before being observed. This method does extend the period of feeding and, no doubt, there will consequently be less bloat than by intermittent pasturing. Nevertheless, the procedure is unsafe for dairy cattle.

Espe, Jacobsen and Cannon (52) have recently suggested that grain be fed before pasturing to prevent bloat. Controlled experiments (125) show that this is an unsound practice.

Some propose to pasture legumes only when free of dew or rain, but bloat has been reported even under these circumstances (122, 36).

The feeding of minerals is frequently employed to prevent bloat. In the authors' opinion, this is merely an exploitation of the stockmen. Admittedly, evidence on the point is lacking.

Some have cut green alfalfa and fed it in the corral. This is apparently an effective preventive, but it is also a costly one as compared to pasturing. It may be effective because the lower stems are consumed with the leafy tops, and thus more irritation is provided than by pasturing.

The status of knowledge regarding the prevention of bloat is very unsatisfactory. Much research is needed to determine the limitations and possibilities of present methods, and to develop new and more effective procedures for safeguarding ruminants on legume pastures. Furthermore, more information is needed on means of preventing chronic bloat—and more particularly, chronic bloat that results from digestive disturbances.

Treatment of Bloat[14]

Very few experimental data are available on the treatment of bloat, largely because it has not been possible to produce bloat at will by feeding. Experimentation is difficult because there are so many spontaneous recoveries from mild bloat; or expensive if one works only with severely bloated animals, because of the high mortality involved.

In the more tractable cases, treatment consists of administering drugs to stop fermentation or to induce belching. As a matter of fact, opinion is di-

[14] This section prepared by H. H. Cole.

vided as to how certain drugs exert their beneficial effect. Healy and Nutter (73) suggest that formaldehyde be given to stop fermentation. No doubt, investigation will show that drugs such as undiluted turpentine in doses of 2 to 4 ounces are fully as effective in relieving bloat and much less toxic.

Some have reported that massage of the left flank is an effective adjunct in reducing mild bloat. Feeding of a scabrous roughage such as Sudan hay apparently has a beneficial effect in certain instances (36). A wooden bit is thought to help in inducing eructation. Some recommend that bloated animals be led up hill or they be placed with their front feet elevated. Many bloated animals will take this position voluntarily if given an opportunity—probably because it facilitates breathing.

In more severe cases it may be found necessary to relieve the animal of excess gas with a stomach tube, trocar canula or, more drastically, by slashing open the rumen with a knife. If time and a stomach tube are available, this should always be the treatment of choice, because of the danger that peritonitis will occur when the peritoneal and ruminal cavities are brought into communication after use of the trocar canula.

Because of the paucity of controlled experiments on the efficacy of various methods of treatment, we wish merely to draw attention to some clinical papers and case reports not cited elsewhere in this review (1, 3, 4, 7, 9, 10, 11, 16, 17, 28, 30, 33, 37, 40, 54, 55, 61, 62, 66, 68, 70, 74, 79, 81, 82, 101, 113, 115, 123, 131, 144, 147, 155, 162, 163, 166, 171, 172, 175, 180, 181, 185, 187, 191, 219).

Theories of Bloat[15]

The early textbooks on livestock diseases describe many of the obsolete theories of bloat. This review is limited to the current theories.

"Excessive Gas Formation" Theory

The theory that bloat in ruminants is due to excessive gas production in the rumen was generally accepted until recently. It is based on the idea that feeds under certain conditions are subject to more rapid fermentation than is normally the case. Veech (202) believed that bloat is due to the consumption of succulent and readily fermentable feeds, which cause the formation of large amounts of gas in the rumen. Similar opinions were held by others (60, 80, 92, 132, 138, 203).

The theory that bloat is due to excessive gas production in the rumen is not supported by results which indicate that the animals have the ability to eliminate far more gas through eruction than is produced in the rumen (35, 157). According to this theory, ruminal gas production should increase when

[15] This section prepared by C. F. Huffman.

animals are turned to pasture or are fed green feed. Schalk (168), however, observed no perceptible excessive intraruminal pressure when animals were turned to pasture. Cole and his associates (35) concluded that bloating on green alfalfa cannot be attributed simply to excessive gas formation, since the tendency is apparently for less gas to be produced from fresh green alfalfa than from alfalfa hay and concentrates. Similar results were reported by Washburn and Brody (204). Jacobson and associates (84), using an *in vitro* method, also reported that fresh green alfalfa does not stimulate gas production any more than fresh green blue grass.

Healy and Nutter (73), using an *in vitro* fermentation method, concluded that the fermentation of the high sugar content of legume blossoms is responsible for bloat; but they did not take into account the fact that fermentation in the rumen occurs on practically all feeds.

The bactericidal and bacteriostatic effects of turpentine and formaldehyde in the treatment of bloat have been used as arguments in favor of the "excessive gas formation" theory. These compounds may exert their value, however, by stimulating the reflex act of belching. Schalk and Amadon (170) showed that coarse or irritating material in the rumen induced the reflex act of rumination, and Cole and his co-workers (35) postulated that eructation is controlled by a similar means.

The evidence points fairly convincingly to the conclusion that excess gas production in the rumen is not the cause of bloat *per se*. On the contrary, most investigators agree that bloat is due to an upset in the normal mechanism for expelling gas. The evidence at hand does not indicate that bloat-provoking feeds result in greater gas formation than feeds not associated with bloat. The proponents of all other theories agree, however, that the rapid formation of gas associated with eating and the consumption of large amounts of feed, with the consequent increased gas production, may be predisposing factors.

"*Excessive Consumption of Dense Feed*" *Theory*

According to this theory, bloat is due to physical obstruction of the cardiac orifice by the rapid ingestion of dense feeds, such as succulent legumes, that prevent the expulsion of gas. This is not a new idea, since for years veterinarians have attributed bloat to a closure of the cardiac orifice and have recommended leading the animal up hill or lowering the rear quarters as a treatment for bloat. The fact that an obstruction in the esophagus, such as an apple or potato, produces bloat is given as evidence supporting this theory.

The effect of blocking the cardiac orifice by placing ruminants on their backs has been studied by several investigators. Bell and Britton (26) postulated that sheep bloat when placed on their backs; but Greeberg and others

(64) and Groenewald (65) were unable to produce bloat in goats and sheep by this procedure.

The modern version of the theory enunciated by Jacobson and his associates (84) is as follows: Ingested roughage is dropped from the cardia into reticulum and anterior compartment of the rumen. Cows that have been without food for 12 to 18 hours may ingest 50 to 100 pounds or more of this succulent feed before resting. This amount of material, if remaining in a relatively compact mass, would tend to force the stomach down against the abdominal floor. Since the only entrance or exit to the rumen and reticulum is at the esophageal groove, anything that would tend to depress the esophageal groove below the level of the fluid in the forestomachs would tend to cause trapping of the gas within the rumen, resulting in bloat. This condition would be aggravated by the cow's lying down after grazing. The gas produced by fermentation would buoy up the ingesta, thus blocking the escape of the gas. Increased pressure would only seal the exit more firmly. The drinking of water might tend to help wash part of this freshly eaten mass back into the rumen; or, on the other hand, if the mass was not dislodged, the water would only make it more difficult for the gas to escape. Many of these suppositions are based upon observations made on the behavior of ruminal ingesta when fermented *in vitro*.

Recently Espe and his co-workers (52) elaborated on this theory. According to them, bloating appears to be entirely a physical problem. Cattle sometimes eat too much legume pasture in too short a time. Feeding grain, dry hay, or silage before putting cattle on legume pastures will prevent overeating and, in turn, prevent bloat. Legume pastures covered with dew or frost are more apt to cause bloat because they permit the cow to eat and swallow the forage faster. The dampened legume may cut down the amount of saliva secreted. These workers believe that decreased saliva secretion increases gas formation. As much gas is formed per pound of bluegrass eaten as per pound of alfalfa. It is only when the opening from the rumen and reticulum is blocked with feed that the cows bloat. Dry cows seldom bloat. It is really the milking cow or growing animals with large appetites that gorge themselves and bloat. These investigators reported that cows ate about three times as much alfalfa pasture as they did bluegrass pasture. No experimental data with bloat cases are presented to support this theory, nor are many of the supporting statements documented. It is mentioned that feeding before pasturing prevents overeating; but Mead and others (125) showed that the feeding of straw before pasturing actually increased the consumption of alfalfa under the particular conditions of their experiment. To our knowledge no experimental evidence is available to indicate that legumes covered with dew or frost are consumed more rapidly.

This theory does not explain the high incidence of bloat by animals on grain alone or the miraculous relief afforded by the administration of either turpentine or formaldehyde to animals bloated on green legumes. Neither does it explain the relief in mild cases of bloat by the feeding of Sudan grass. Dougherty (43) pumped water into the rumen and elevated the posterior end of the animal, thus stopping belching. Although the animal showed some uneasiness, it was in no distress at any time—a further indication that bloat is not due to stoppage alone. Most likely, however, the rate of gas formation in the rumen of Dougherty's animal was at a low ebb.

The evidence for this theory rests largely upon the behavior of ruminal ingesta fermented *in vitro*. Jacobson and his co-workers' report that legumes are consumed more rapidly than certain grasses is also a supporting argument; but Cole and Kleiber (unpublished data) found a reversed relation regarding the consumption of alfalfa and Sudan pasture with a cow which bloated regularly on alfalfa but not on Sudan. (See the section "Expulsion of Gas.") Though bloat is more common among fast eaters, it has been observed among slow ones (164, 125).

"Toxic Gas" Theory

This theory postulates that legume plants, which most often cause bloat, contain high percentages of protein, which produce an excess of toxic gases in the rumen. These gases, singly or combined, partly paralyze the rumen and so prevent belching.

Dougherty (43) reported that only mild symptoms were produced by increasing intra-ruminal pressure by air, hydrogen, or methane. Carbon dioxide, however, produced extreme dyspnea. No other symptoms were observed. The chemical analyses of the rumen gases before and after feeding freshly cut Ladino clover indicated an increase in the carbon monoxide content. Bloat was produced by pumping exhaust gas into the rumen and increasing the pressure in the rumen with carbon dioxide and methane. The carbon monoxide content of the blood was 80 to 90 percent saturated. Bloat, associated with increased blood carbon monoxide was produced in three other cows by this method. As intraruminal pressure increased, the rate of absorption increased. Conceivably the greater area of ruminal mucosa exposed, results in a greater rate of absorption. Dougherty concluded that carbon monoxide, when present in the rumen in very low concentrations, will produce marked symptoms when the intraruminal pressure is increased by means of other gases common to the rumen. The intraruminal pressure in this case may not be extreme. For a rational explanation of this observation, more research is necessary. Carbon monoxide was found in the rumen in an appreciable amount in two experimental cows fed freshly cut Ladino clover.

Dougherty (44) reported that the hydrogen sulfide content of the ingesta was increased by feeding good alfalfa hay and freshly cut Ladino clover and alfalfa. Carbon monoxide was not correlated with feed changes. The injection of small amounts of hydrogen sulfide and carbon monoxide into the rumen was toxic when intraruminal pressure was increased by air insufflation. Both gases caused a distinct paralysis of the rumen when sufficient concentration was reached in the rumen. In both *in vitro* and *in vivo* studies cystine, methionine, and inorganic sulphur caused an increase in hydrogen sulfide production. Appreciable amounts of hydrogen sulfide were found in ruminal gas and ingesta from a heifer that died of bloat. This animal had been dead a few hours. Ingesta and gas samples taken shortly after death from three steers that had died of acute bloat showed relatively high concentration of hydrogen sulfide (45). Incidentally, the ingesta from these animals also contained relatively large amounts of histamine.

Dougherty and his co-workers (47), who reviewed the literature pertaining to hydrogen sulfide poisoning, advanced the hypothesis that hydrogen sulfide inactivates or inhibits some enzyme system and paralyzes the respiratory system. The evidence indicates that relatively small amounts of hydrogen sulfide are required to cause death. Intoxication develops with extreme rapidity, but animals recover rapidly under treatment even when prostrated.

The "toxic gas" theory was supported by Olson (140), who found appreciable amounts of carbon monoxide in the ruminal gases from cows which had bloated on sweet clover and alfalfa. Olson concluded that the percentage of gases indicated by the analyses do not in themselves point to the cause of bloat, or the cause of death from bloat. Possibly the rapid formation and absorption of gases when legumes are eaten are due to a series of factors, none of which will cause bloat or death in themselves. Carbon monoxide and hydrogen sulfide had no recognizable toxic effect unless accompanied by increased intraruminal pressure. The other gases present in the rumen gas had no effect on the animal; yet these gases may perhaps induce the conditions that make toxic gases fatal, by increasing the intraruminal pressure or inhibiting the normal functioning of the rumen.

Olson (143) recently reported that both carbon monoxide and hydrogen sulfide are present in rumen gas of normal animals, and that hydrogen sulfide is greatly increased in case of bloat. He cited the work of Alway (5) and Painter (146), who showed that the protein of legumes is higher in sulphur than the protein of nonlegumes; and the leaves and finer stems of legumes, the parts eaten by grazing animals, are relatively higher in sulphur than the stems of the same plant (146). Olson used four cows to study the hydrogen sulfide content of rumen gas when the animals were given fresh green alfalfa in the barn, on pasture, and on dry feed. The results showed a higher per-

centage of hydrogen sulfide when the cows were stable-fed freshly cut green alfalfa than when they were on dry feed or grazed on alfalfa starting to blossom. The explanation of the failure of the grazing animals to show more hydrygen sulfide in the ruminal gas was that the cows grazed on the dandelions and grasses more than on alfalfa.

In answer to the question why legumes can be safely pastured on certain farms some years but will cause bloat in other years, Olson (143) referred to his work, in which milk cows were pastured on both alfalfa and sweet-clover experimental plots for seven consecutive years without a single case of bloat. But in a period of abnormal rainfall, cows bloated to such an extent that grazing on these plots had to be discontinued. Olson postulated that the reason cows bloat in some years and not in others on the same legume pasture may be explained by variation in rainfall. With heavier rainfall the plants will grow more rapidly and contain more sulphur. If hydrogen sulfide is produced in the rumen from the sulphur content of high-protein plants, and if these plants depend upon the sulphur content of the soil, it is possible to explain why cows may bloat on some legume pastures and not on others. They will bloat on plants from soil containing the larger amount of sulphur. McIntosh (124) also attributed bloat to soil conditions, but did not incriminate any single factor.

Olson's experiments (143) indicated that when animals bloat, there is considerable increase in hydrogen sulfide gas as compared with animals on dry feed. The amount of hydrogen sulfide gas in the rumen of bloated animals may be 10 to 20 times as much as from the rumen of animals on dry feed. He cited a fact long known to toxicologists—namely, that hydrogen sulfide gas is highly toxic. Small amounts of this gas when absorbed into the blood stream are fatal. Alfalfa at certain stages of growth is very high in sulphur. This seems to indicate that hydrogen sulfide gas may play a significant role in the cause of death of bovines from bloat.

Kleiber and others (94) studied the hydrogen sulfide content of ruminal gas from two bloated cows and three normal cows on alfalfa pasture. There was no significant difference in the hydrogen sulfide content of these samples. The hydrogen sulfide content of the ruminal gas from a cow fed fine alfalfa hay and two cows fed Sudan grass the night before being turned out to pasture was studied. The values fell within the normal range. There was no relation between hydrogen sulfide concentration in the rumen and bloat.

It is difficult to decide from the papers of Dougherty and Olson whether they were postulating that toxic gases were responsible for bloat or that these substances were merely responsible for death after bloat had developed. Dougherty (personal communication, 1944) makes his present position clear, however, as follows: "I do not believe that toxic gases are responsible

for such cessation of motility. (At one time I thought that CO might have such an influence, but later work did not substantiate such belief.) I have only contended that they (perhaps mainly H_2S) play an important part in causing death."

Olson (143) apparently felt that hydrogen sulfide was responsible for bloat as well as for death following bloat: "The percent of gases except sulfide and methane remain about the same in normal and bloated animals. The assumption therefore is that hydrogen sulfide, a highly toxic gas, paralyzes the rumen and is then absorbed. When a small percent of hydrogen sulfide gas enters the systemic blood, immediate death results."

Until hydrogen sulfide is shown to be greater in *living* bloated animals than in normal animals, the theory that it causes bloat lacks substantiation. Evidence available (94) is to the contrary. Considering the very appreciable amounts of hydrogen sulfide present in the rumen at all times and the toxic effects when it is artificially introduced into the rumen, hydrogen sulfide may well be a contributing factor in death of bloated animals.

Saponin Theory

McCandlish (120) claimed that the chemical substance in clover or in potatoes which appears to cause bloat is saponin. The ill effects were induced presumably, not by extra production of gas in the rumen, but by causing the retention of the gas already formed. Olson (142) also referred to the saponin theory as an explanation of the bloat associated with froth and foam in the rumen.

A point at issue here is if the frothing is actually the cause of bloat. It is true that the exuding of frothy material from an opened rumen or from the canula tapping the rumen of bloated cows has been repeatedly observed. Amadon (personal communication) concludes from his experiences that most cases of bloat on green legumes are of the frothy type; Kick, Gerlaugh and Schalk (93) believe that frothiness is a factor in bloat of steers fed corn in the feed lot. Cole and his co-workers (unpublished data), on the other hand, have tapped a number of cows bloated on alfalfa pasture without encountering a case in which the free air space was absent. The fact that often no free gas but, rather, frothy ingesta, escapes when the rumen is tapped is regarded by many as proof that free gas is not present. The cause, conceivably, may be the fact that the free air space is difficult to tap when the rumen is excessively filled. If a free air space exists, it is difficult to understand why frothing would cause bloat. At the moment at which belching occurs there is a dilation of the reticulum and anterior rumen, thus tending to free the cardia for passage of gas into the esophagus. In other words, one cannot think of the rumen and reticulum as organs of constant size. Their size is modified not only by active contractions but also by changes in muscle tonus.

Quin (157) has proposed a modified saponin theory based upon his expermental and clinical findings. He writes as follows: "The condition arises through a combination of (a) the presence of characteristic pseudo-yeast cells in the rumen, (b) the rapid consumption of fresh lucerne leaves showing an elevated sugar content, (c) the establishment of an aerobic state in the rumen associated with rapid swallowing of food thus producing excessive oxidation of sugar and gas formation, (d) the presence of saponin in lucerne which by increasing surface tension and the tendency towards foam formation, impedes the escape of gas from the ruminal mass." Quin confirmed Jacobson's finding (83) that a saponin could be isolated from alfalfa having strong foam-producing qualities and noted that fresh ruminal ingesta drawn from sheep on alfalfa pasture had a greater tendency to foam than ingesta from animals on other diets. He noted, further, that ingesta taken from sheep bloating on alfalfa "consisted of a strongly foaming, frothy mass."

Jacobson (83) fed 19 grams of alfalfa saponin to a sheep without any ill effects. Although saponin has been detected in many feeds, quantitative data supporting this theory are meager.

"*Physical Deficiency*" *Theory*

The "lack of coarse roughage" theory as the cause of bloat was advanced by Cole and his co-workers (35). According to this theory, most bloat on green legumes results from the absence of stimuli necessary to initiate belching. When the mechanism to induce belching is normal, then the cow can belch not only the maximum amount of gas formed under natural conditions, but even greater amounts. The chronic bloater is explained as having a higher threshold of irritability. Even coarse roughage in the rumen may be insufficient, in such cases, to stimulate belching.

The same writers (36, 125) recognized later, however, that chronic bloat may be due to ruminal atony. They cite a case of bloat that was associated with peritonitis even though hay and grain were being fed. They remark that chronic bloat in calves with digestive disturbances may be due to rumen immotility, but conclude that bloat on legume pasture is due largely to a lack of sufficient irritating roughage in the rumen. They do not mention the well-recognized fact that enlargement of the mediastinal lymph glands or other tumorous growths pressing upon the esophagus may result in chronic tympany (25).

This "physical deficiency" theory is supported by the fact that bloat can be produced or prevented on such a basis. Conclusive proof that the physical condition of the feed is an etiological factor in bloat is provided by the experiment in which the same workers compared the incidence of bloat on ground hay and grain as compared with whole hay and grain. A greater incidence of

bloat occurred when ground hay and grain were fed. Since the two diets had the same chemical composition, the difference between them must have been due to a change in the physical nature of the diet. This does not prove the second part of the theory, however—namely, that the lack of coarse material in the rumen results in insufficient stimulus of nerve fibers (terminating in the wall of the rumen or esophagus) to elicit the eructation reflex. Only indirect evidence has been submitted for this second point. The same writers (36) showed that a rough correlation existed between rumination and belching. In other words, on rations in which belching was deficient and bloat consequently occurred, there was likewise a lack of normal rumination. Schalk and Amadon (170) provided conclusive evidence that rumination depends upon the stimulation of nerve fibers in the rumen by coarse roughage. That belching is a reflex rather than a voluntary act in the ruminant is indicated by the fact that involuntary muscles are involved in the act.

Although this "physical deficiency" theory for bloat on green legumes is backed by more experimental evidence than other current theories, the following evidence should be submitted before it is accepted in the realm of fact: (1) it should be proved that stimulation of nerve fibres in the esophagus or rumen elicites the act of eructation; (2) it should be shown that the addition of a chemically inert scabrous material to a bloat-provoking diet will prevent bloat. Cole and co-workers have shown that the addition of scabrous material such as Sudan hay or Sudan grass pasture will prevent bloat, but there is no proof that chemical constituents of Sudan are not involved. They have shown, furthermore, that turpentine, an irritating material, will relieve bloat. Almost certainly, belching is facilitated by the administration of turpentine; but the exact mode of action involved has not been determined.

"*Abnormal Ruminal Microflora or Microfauna*" *Theory*

The possibility that bloat may be due to a lack of balance of microorganisms normally found in the rumen or to the presence of an abnormal type has been suggested. Koffman (100) studied the rumen fauna of normal sheep and of sheep affected with bloat. In the latter there was a decrease in the number of large protozoa in the ingesta. Koffman suggested that this change may have resulted in a greater number of gas producers.

Quin (156) suggested the possibility that bloat may be due to diet which is unbalanced for the ruminal microflora. He also postulated that a deficiency of any essential nutrients should cause, in the microflora, some form of disturbance in which either the population as a whole or some of its individual members are subjected to a depression. Should such a disturbance be severe enough, obviously the various processes normally occurring in the fore-

stomachs might be replaced by a series of pathological conditions expressed in symptoms such as atony, stasis, bloat, or diarrhea.

Quin (157) concluded that clinically acute bloat represents an integral of the normal processes associated with sugar metabolism in the fore stomachs of ruminants. Pseudo-yeast cells (schizosaccharomyces species) in the rumen immediately assimilate and store the sugar, mainly as glycogen; the remaining sugar is exposed to a fulminating oxidation, resulting in the production of large volumes of gas, comprised chiefly of carbon dioxide. He reported that alfalfa contains more sugar in the afternoon than in the morning; the sugar content parallels the occurrence of bloat. According to him, since starvation readily leads to depletion of the glycogen reserves within the yeast cells of the rumen, one must safeguard the ruminant against periods of acute hunger, especially where green succulent alfalfa constitutes the major item in the ration. Under such conditions, the ingestion of fresh green alfalfa is immediately associated with an accentuated phase in the assimilation and oxidation of the plant sugars by the starving yeast cells, and thus the tendency toward acute bloat is established. Alternately, the regular feeding of carbohydrate supplements with the legume ration keeps the energy demands of the yeast cells more or less satisfied. The sugars in the alfalfa are therefore not metabolized at the same dangerously high speed, and gas and foam production are controlled. This type of bloat has been described above.

The possibility that a yeast may be present in the rumen was also suggested by Quin (156), Johnson and others (85), and Begg (25). The theory advanced by Quin may explain the frothy type of bloat but there is no evidence that the sugar content of legumes producing this type of bloat is higher than normal. Ordinarily, fresh grasses contain much more sugar than fresh legumes (211). The role advanced for saponin in bloat is without experimental support.

Discussion of Theories

Excess gas production in the rumen is not the cause of bloat *per se*, since the ruminant can eruct more gas than is likely to be produced. According to the evidence at hand, there are several causes of bloat: 1. Complete or partial obstruction of the esophagus. Substances such as tubers, lodging in the esophagus, cause acute tympany. The esophagus may be partially obstructed by pressure of tumorous growths, such as enlargement of the mediastinal lymph glands, upon the esophagus. The usual result is chronic tympany. 2. Ruminal atony. Eructation occurs when the rumen is in an active state of contraction (208, 35). As Amadon (personal communication) has observed, ruminal contraction is essential in moving the air pocket an-

teriorly to the cardiac orifice. Apparently, therefore, tympany associated with digestive disturbances may be due to ruminal atony. Begg (25) asserts that this is the case; Cole and his co-workers cite an instance of tympany (following peritonitis) which they believe was caused by ruminal atony. Mead and others (125) have postulated that bloat occurring after animals have been on a grain ration for long periods may be due to ruminal atony. Histamine, phosgene, and other toxic substances administered to ruminants probably cause tympany by producing ruminal atony. Although Dougherty found histamine in rumen ingesta, he does not attempt to explain bloat on green legumes upon this basis; rather, he believes that histamine may be a contributing factor in death of bloated animals. 3. Frothy ingesta. If frothiness of the ingesta can prevent the escape of free gas from the rumin, saponins or other colloidal substances must be responsible for this type of bloat. Attempts to produce bloat by feeding saponins have not been successful. 4. Defective eructation mechanism. It seems likely that most bloat on green legumes may be due to a defective belching mechanism. The evidence indicates that ruminal contractions, even after mild bloat is established, are energetic under these conditions. Jacobson and his associates' theory that eructation is prevented by a "piling up" of freshly ingested legumes is not supported by direct experimental evidence. Although Olson and Dougherty have shown that more hydrogen sulfide is formed on green legumes than on dry feeds, the finding of similar amounts of it in rumen gas from living cows on bloat-provoking and non-bloat-provoking diets does not support the view that it is responsible for the defective eructation mechanism. Very likely, however, hydrogen sulfide and other toxic gases and substances in solution may be contributing factors in the death of bloated animals. The view of Cole and his co-workers that there is a lack of sufficient coarse roughage to induce eructation has the support of experimental evidence. As has been shown, the physical condition of the feed may be an etiological factor in bloat. There is circumstantial evidence that eructation, like rumination, depends upon coarse roughage in the diet. Final proof is lacking, however, to show that bloat on green legumes depends upon a lack of scabrous material to elicit the eructation reflex.

All the current theories of bloat emphasize the role of gas eructation. One should not lose sight of more remote possibilities, such as diminished gas absorption through the rumen mucosa. It has been demonstrtaed that large amounts of carbon dioxide and methane can be absorbed through the rumen wall (43, 96, 204, 59). Factors affecting this route of escape may contribute to bloat.

Any factor that stimulates circulation in the wall of the rumen may increase the absorption of carbon dioxide and methane through the rumen

wall. According to Kato (89), Kader (87), and Schoen (174) a defect in the circulation of the mucous membrane of the intestine could have more to do with the production of flatulence than any other factor. As Schoen pointed out, the chief factor in lessening the amount of absorption of gas from the intestine is a lowering of the tonus of the intestinal wall. Further information on the exchange of gases between the rumen and the blood is desirable, not only from the standpoint of bloat but also for a better understanding of metabolic activity within the rumen.

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DISTILLERS' FEED PRODUCTS IN RATIONS FOR GROWING PIGS

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Introduction

A COMPREHENSIVE review of the literature has been published by Bauernfiend and Boruff (1944, 1944a) concerning the value of corn distillers' dried grains and corn distillers' dried solubles in rations for growing pigs and lactating sows. It is evident from this report that corn distillers' dried solubles contain many of the B complex vitamins which are necessary in promoting good growth and development for the growing pig. This product was also reported to be of practical significance in diets composed largely of vegetable protein concentrates.

In this review corn distillers' dried grains were reported to be of value in supplementing the grain mixture of pigs fed either in drylot or on pasture. At a 12 percent level, this distillers' feed product was found to enhance the nutritive qualities of a ration containing ground corn, wheat flour middlings, soybean oil meal, tankage, fish meal, minerals and cod liver oil for pigs from the creep-feeding periods until marketing.

During the past two years, the author has carried out studies to confirm the value of distillers' feed products in growing pig rations containing a high level of animal protein. In addition, it was the object of these studies to determine the most efficient use of distillers' feed products in various types of rations for growing pigs which contained a low level of animal protein. Most of the animal protein products contain a good quality protein and a fairly high level of the B-complex vitamins. The protein of the distillers' feed products is derived principally from grain, and to a certain extent from the yeast used in the fermentation process. Since about 25 percent of this product is yeast, it was the author's opinion that the distillers' feed products would be of greatest value in practical growing rations for pigs as a vitamin supplement either in concentrates containing a small amount of animal protein or concentrates in which the protein is derived entirely from vegetable sources.

Experimental Procedure and Results

In trials 1 and 2 equal numbers of eight-week-old male and female pigs of mixed breeding (Hampshire and Poland China) were purchased on the open market for use as experimental animals. Purebred Duroc-Jersey pigs were used in trials 3 and 4. The Duroc pigs were creep fed from two to six weeks

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of age in drylot with concrete floor, at which time they were weaned and started on the experimental diets. All pigs were vaccinated for septicemia and cho'era.

At the beginning of each experiment the pigs were ear-tagged, weighed and allotted to the various pens according to weight and sex. Ten pigs were used in each pen. Drylot pens with adequate shelter were used for all trials.

In trial 1 the pigs were self-fed the protein supplement-grain mixture, while in trials 2, 3 and 4 the free choice method of feeding the supplement and grain was used. Block salt was kept before the animals at all times.

Trial 1

The chemical analyses of distillers' dried grain and distillers' dried solubles, presented in table 1, indicate that these two ingredients would be of value as a source of protein and the B complex vitamins in rations for growing pigs.

TABLE 1. CHEMICAL AND VITAMIN CONTENT OF CORN DISTILLERS' FEED PRODUCTS

Item	Solubles	Dried grain
Protein (percent)	30	28
Fat (percent)	10–12	9
Fiber (percent)	3	6–8
Ash (percent)	6	4
Riboflavin (micrograms per gram)	15–20	6
Thiamin (micrograms per gram)	11–15	3–4
Pantothenic Acid (micrograms per gram)	25–30	10–13
Niacin (micrograms per gram)	170	70–90
Pyridoxine (micrograms per gram)	8–10	—
Choline (micrograms per gram)	5,800	4,000–5,000
Biotin (micrograms per gram)	.3–.4	.1–.2

Trial 1 was designed to determine the relative value of the proteins of tankage, distillers' dried solubles and distillers' dried grains in rations for growing pigs. In formulating the different rations used in this experiment (table 2) it was deemed necessary that an 18 percent crude protein diet be fed until the pigs attained approximately 100 lbs. in weight or for the first eight weeks of the experimental period. During the eight to twelve week period, the protein content of the rations fed was reduced to 12 percent.

From the data presented in table 3, it is evident that the ration containing the high level of tankage produced the most rapid and economical gains (lot 1). Distillers' dried solubles and distillers' dried grains followed in order as to their value in rations for growing pigs.

TABLE 2. PERCENTAGE COMPOSITION OF DIETS FED TO GROWING PIGS IN TRIAL 1

Ingredients	Periods and lot numbers					
	1–8 week period			8–12 week period		
	1	2	3	1	2	3
	percent	percent	percent	percent	percent	percent
Ground yellow corn	73.0	59.0	59.0	82.0	75.0	75.0
Tankage (60 percent protein)	18.0	7.0	7.0	5.0	—	—
Distillers' dried solubles	—	25.0	—	—	12.0	—
Distillers' dried grains (with solubles)	—	—	25.0	—	—	12.0
Alfalfa meal	6.0	6.0	6.0	10.0	10.0	10.0
Steamed bone meal	3.0	3.0	3.0	3.0	3.0	3.0
Total	100.0	100.0	100.0	100.0	100.0	100.0
Percentage protein	18.7	19.3	18.7	12.3	12.7	12.00
Percentage calcium	2.22	1.44	1.44	1.14	.99	.99
Percentage phosphorus	1.19	.87	.87	.66	.66	.66

No deficiencies were noted among the pigs in the various lots. Even though no difficulty was encountered with pigs refusing to eat, it was evident that lot 1 consumed the most feed.

TABLE 3. SUMMARY OF DATA FROM TRIAL 1

Item	Lot number		
	1	2	3
Experimental time (days)	84	84	84
Av. initial weight (lbs.)	32.1	32.5	29.2
Av. final weight (lbs.)	145.4	104.7	95.6
Av. daily gain (lbs.)	1.34	.86	.79
Av. daily gain (lbs.) 1–8 wks.	1.11	.90	.78
Av. daily gain (lbs.) 8–12 wks.	1.81	.77	.79
Av. total feed consumed (lbs.)	370	295	290
Lbs. feed per 100 lbs. gain	328	408	563
Feed cost per 100 lbs. gain* (dollars)	6.84	9.49	10.61

* Market feed prices used as of October 1, 1942.

The greatest gain was obtained from those pigs fed tankage (lot 1), and there was very little difference between the gains of those pigs fed the distillers' feed products (lots 2 and 3). Lot 2 made slightly better gains than lot 3; especially during the first eight-week period. This was in all probability

owing to the greater concentration of yeast protein and lower fiber content of the distillers' dried solubles which would increase the biological value of this product for growing pigs.

On the basis of the data presented, distillers' dried grains are of little value as a protein concentrate for the growing pig. This is partially attributed to the lower biological value of the protein and the relatively high fiber content of the dried grains in comparison to tankage.

The data also show that tankage excells distillers' dried solubles as a major source of protein for the growing pig. Here, again, the fact arises that even though distillers' dried solubles contain 25 percent by weight yeast, the total protein is not as high biologically as in tankage.

Trial 2

The results of trial 1 showed that the distillers' feed products could not be used effectively as the major source of protein for the growing pig. However, vitamin assays and chick feeding trials have shown that distillers' dried solubles are a good source of the B complex vitamins. Consequently, if distillers' dried solubles were incorporated as a vitamin supplement with good quality vegetable protein concentrates which are low in the B-complex vitamins, it was considered possible to formulate a growing pig supplement containing distillers' feed products which would produce rapid and economical gains.

TABLE 4. PERCENTAGE COMPOSITION OF SUPPLEMENTS FED TO PIGS IN TRIAL 2

Ingredients	Lot No.		
	1	2	3
	percent	*percent*	*percent*
Tankage	35.7	17.2	—
Soybean oil meal	35.7	28.6	51.2
Distillers' dried solubles	—	28.6	24.4
Alfalfa meal	21.5	17.0	14.6
Steamed bone meal	7.1	8.6	9.8
Total	100.0	100.0	100.0
Percentage protein	39.1	33.4	32.8
Percentage calcium	5.388	4.588	3.703
Percentage phosphorus	2.730	2.635	2.226

The supplements fed the pigs in trial 2 are presented in table 4. Lot 1 (control) was fed a supplement containing equal amounts of tankage and soy-

bean oil meal, plus alfalfa meal and steamed bone meal. Distillers' dried solubles were used to replace a portion of the tankage, soybean oil meal and alfalfa meal in the supplement fed lot 2. The supplement fed lot 3 contained no tankage, and the concentrate was designed to contain at least 32 percent protein from the vegetable sources.

There was very little difference between lots 1 and 2 insofar as the daily gain, feed efficiency, and pounds of protein required per 100 lbs. of gain were concerned (table 5). Actually, there was only 0.2 percent difference in the amount of protein consumed as calculated from the total amounts of corn and supplement consumed. Lot 2 consumed more concentrate than lot 1, because there was 5.7 percent difference between the two concentrates in protein content, and consequently, more of the lower protein concentrate was required to satisfy the needs of the growing pig.

It is evident from table 5 that the low animal protein supplement afforded the most rapid rate of gain during the first eight weeks or until the pigs attained 90 to 100 lbs. in weight. But, during the eight to twelve week period, there was a tendency for the average gains of those pigs fed the high and low animal protein supplements to become closer. The average gain of lot 3 was consistently lower than those of lots 1 and 2.

TABLE 5. SUMMARY OF DATA FROM TRIAL 2

Item	Lot number		
	1	2	3
Experimental time (days)	112	112	112
Av. initial weight (lbs.)	41.0	41.0	40.0
Av. final weight (lbs.)	193.3	199.3	182.5
Av. gain (lbs.)	152.3	158.3	142.5
Av. daily gain (lbs.)	1.36	1.41	1.27
Av. daily gain (lbs.) 1–8 wk.	.92	1.12	.87
Av. daily gain (lbs.) 8–16 wk.	1.80	1.71	1.68
Feed per 100 lbs. of gain (lbs.)	448	452	490
Corn (lbs.)	374	360	399
Supplement (lbs.)	74	92	91
Lbs. of protein	62.7	62.8	67.2
Feed cost per 100 lbs. gain* (dollars)	10.80	11.07	11.56

* Market price of feedstuffs as of June 2, 1943.

The amount of supplement consumed per 100 lbs. of gain was about equal for lots 2 and 3, but lot 3 consumed 42 lbs. more corn on the average for the same gain than did lot 2. Because lot 3 received an all-vegetable protein diet, the increase in feed and protein consumption may be explained on the basis that the level of essential amino acids may have been suboptimal for rapid

gains. It is possible that a greater variety of vegetable proteins would raise the level of these essential constituents and result in a more rapid rate of gain.

Trial 3

From the results of the previous trials, it became evident that two important factors were required to complete the problem concerning the development of a growing pig supplement containing distillers' dried solubles. First, it was deemed necessary that a standard control supplement was needed to compare the performances of the all-vegetable and low animal protein supplements; second, a greater variety of protein sources was required for optimum performance of the all-vegetable supplement.

The trio mixture was selected as the control diet in this experiment. According to Morrison (1937) this ration would produce almost phenomenal results for growing pigs. Pigs weighing 53 lbs. were fed the trio mixture in

TABLE 6. PERCENTAGE COMPOSITION OF SUPPLEMENTS FED IN TRIAL 3

Item	Lot number		
	1	2	3
	percent	*percent*	*percent*
Tankage	50.0	9.0	—
Linseed meal	25.0	—	25.0
Soybean oil meal	—	48.0	32.0
Distillers' dried solubles	—	18.0	18.0
Alfalfa meal	25.0	18.0	18.0
Steamed bone meal	—	7.0	7.0
Total	100.0	100.0	100.0
Percentage protein	42.2	36.8	31.4
Percentage calcium	3.688	3.052	2.490
Percentage phosphorus	1.738	1.517	1.422

drylot and gained on the average of 1.28 lbs. per day. An average of 351 lbs. of corn and 63 lbs. of supplement were required per 100 lbs. of gain. Lot 2 (table 6) was fed a supplement designed to contain 9 percent tankage which would supply about 15 percent of the protein in the supplement or about 5 percent of the protein in the total diet. The balance of the protein was derived from distillers' dried solubles, soybean oil meal and alfalfa meal. The supplement fed lot 3 was similar to supplement 3 of trial 2, except that a portion of the distillers' dried solubles and soybean oil meal was replaced with linseed meal. Due to the high concentration of protein in tankage, a wide range of protein values resulted among these three diets.

The resulting data of trial 3, as presented in table 7, show that there was not a great deal of difference in the final weights or average gains of pigs fed the three supplements. However, during the first four-week period of the experiment, the pigs in lots 2 and 3 made a greater gain than did those pigs in lot 1. This same condition was noted during the four to eight week period in lot 2 which indicated that supplement 2 contained greater quantities of the nutrients necessary to promote rapid growth for pigs during this early period.

TABLE 7. SUMMARY OF DATA FROM TRIAL 3

Item	Lot number		
	1	2	3
Experimental time (days)	126	126	126
Av. initial weight (lbs.)	25.0	24.7	24.8
Av. final weight (lbs.)	188.2	185.4	181.5
Av. gain (lbs.)	163.2	160.7	156.7
Av. daily gain (lbs.)	1.30	1.28	1.25
Av. daily gain (lbs.) 1–4 wks.	.46	.52	.55
Av. daily gain (lbs.) 4–8 wks.	.94	1.00	.85
Feed per 100 lbs. gain (lbs.)	392	391	406
Corn (lbs.)	302	283	287
Supplement (lbs.)	90	108	119
Protein (lbs.)	66.7	66.3	64.6
Feed cost per 100 lbs. of gain* (dollars)	9.68	9.58	9.89

* Market price of feedstuffs as of February 15, 1944.

The data show that the pigs in lot 1 consumed the most feed. However, the pigs in lots 1 and 2 utilized their feed with about equal efficiency, while a little more feed was required per unit of gain in lot 3. When breaking down the values representing the amount of feed required per 100 lbs of gain, it was found that from 18 to 27 lbs. less supplement was consumed by lot 1. This can be readily explained by the fact that supplement 1 contained 42.2 percent protein, while supplements 2 and 3 contained 36.8 percent and 31.4 percent protein respectively. The amount of protein required per 100 lbs. of gain was practically the same for lots 1 and 2 and a little lower for lot 3.

It is apparent from the data presented that the pigs fed the trio mixture in this trial consumed 27 lbs. more supplement but 49 lbs. less corn per 100 lbs. of gain than those pigs reported by Morrison (1937). The average daily gains were about equal.

Trial 4

In order to obtain further information concerning the performance of all-vegetable and low animal protein supplements for growing pigs, an experi-

ment was designed to compare the supplements used in trial 3 under both pasture and drylot conditions. It was recognized that a feed saving of from 7 to 20 percent may be procured through the use of good pasture, however, it was not known to what extent pasture would supplement these diets.

The supplements fed lots 1, 2, and 3 in drylot were identical to those supplements fed in trial 3 (table 6). Lots 4, 5 and 6 (table 8) had access to pasture consisting of alfalfa, lespedeza, orchard grass and brome grass. The pigs obtaining pasture received no alfalfa meal in their diets; therefore that portion of the diet which was alloted to alfalfa meal was divided among the other vegetable proteins. Consequently, the protein content of these concentrates was approximately 5 per cent higher than those fed the drylot pigs.

TABLE 8. PERCENTAGE COMPOSITION OF SUPPLEMENTS FED PIGS ON PASTURE IN TRIAL 4

Ingredients	Lot Number		
	4	5	6
	percent	*percent*	*percent*
Tankage	62.5	9.0	—
Soybean oil meal	—	57.0	38.0
Linseed meal	37.5	—	31.0
Distillers' dried solubles	—	27.0	24.0
Steamed bone meal	—	7.0	7.0
Total	100.0	100.0	100.0
Percentage protein	48.2	41.6	37.8
Percentage calcium	4.645	2.814	2.225
Percentage phosphorus	3.227	1.711	1.466

During the fifth week of this trial, one male and female pig in lot 5 died of unknown causes. Since the experiment was at an early stage, two female pigs of about the same weight were substituted. At the twelve-week period, two female pigs in lot 3 showed signs of unthriftiness, but they exhibited no nutritional deficiencies. When excluding these animals from the data, the average weight of the lot is 154.6 lbs., which would increase the average daily gain to 1.04 lbs.

The value of pasture in reducing the cost of a unit of gain is quite significant (table 9). It cost 12 percent less to feed the high-animal protein supplement, 21 percent less for the low-animal protein supplement, and 27 percent less for the all-vegetable protein supplement on pasture than in drylot. There was very little difference between the cost per 100 pounds of gain when the high and low-animal protein supplements were fed in drylot, but the all-vegetable protein supplement cost about 11 percent more. When

pasture was used to supplement these concentrates, the low-animal protein supplement produced a unit of gain at a 10 percent lower cost than the high-animal protein supplement, but only 4 percent lower than the all-vegetable protein supplement.

With the exception of lot 3, there was very little difference in the average gains among the various lots. Lots 4 and 6 did not make quite as rapid a gain during the early part of the experiment as the other lots, but lot 5 made about the same rate of gain as lots 1 and 2.

TABLE 9. SUMMARY OF DATA FROM TRIAL 4

Item	Lot number					
	1	2	3	4	5	6
Experimental time (days)	112	112	112	112	112	112
Av. initial weight (lbs.)	38.8	38.7	38.1	38.9	38.3	39.0
Av. final weight (lbs.)	172.2	175.3	143.2	170.2	178.2	169.4
Av. total gain (lbs.)	133.4	136.6	105.1	131.3	139.9	130.4
Av. daily gain (lbs.)	1.19	1.22	0.94	1.17	1.25	1.17
Feed per 100 lbs. of gain (lbs.)	387	395	446	351	315	333
Corn (lbs.)	317	325	360	328	285	309
Supplement (lbs.)	70	70	86	23	30	24
Protein (lbs.)	59.2	56.4	60.8	41.9	38.3	33.6
Estimated lbs. of protein per 100 lbs. of gain saved by pasture	—	—	—	17.3	18.1	27.2
Cost of corn per 100 lbs. (dollars)	2.56	2.56	2.56	2.56	2.56	2.56
Cost of supplement per 100 lbs. (dollars)	3.37	3.11	2.98	3.49	3.18	3.06
Feed cost per 100 lbs. gain (dollars)	10.46	10.49	11.79	9.20	8.26	8.64

The performance of the all-vegetable protein diet in this trial did not equal that of trial 3. It was observed that during the last forty days of the experiment, the pigs fed the all-vegetable protein supplement on pasture rooted the ground to a greater extent than did those receiving animal protein in their diet. Owing to the fact that the pigs fed on pasture consumed less than half the proportion of supplement than did those fed in drylot, it is evident that the all-vegetable protein supplement may contain a sub-optimal level of calcium and phosphorus. This condition would also tend to cause an excessive amount of rooting and be conducive to slower gains. Experiments are now in progress to investigate this point in an effort to improve the performance of this type of ration.

Summary

Distillers' dried solubles and distillers' dried grains were compared with tankage as a source of protein in rations for growing pigs from 30 lbs. to approximately 145 lbs. It is evident that the distillers' feed products did not

contain all of the essential amino acids at a level required to promote a satisfactory rate of growth. Since the lot fed the distillers' dried grain made slower gains than the one fed distillers' dried solubles, it is apparent that the added fiber was also a factor hindering satisfactory gains.

When distillers' dried solubles were used as a vitamin supplement in concentrates containing a small quantity of animal protein or protein entirely from the vegetable sources, practical rations for growing pigs were formulated which produced results equal to the control ration (Wisconsin trio mixture) and at its equivalent costs.

The addition of linseed meal to the all-vegetable protein concentrate increased the value of this concentrate as measured by the rate of growth and efficiency of gain.

Very little difference was noted between the high- and low-animal protein diets in relation to performance. During the early period or up to the time the pigs attained 100 lbs. in weight, the low level animal protein diet produced slightly faster gains. There was indirect evidence that an increase in the calcium and phosphorus level in the all-vegetable protein supplement when fed on pasture would result in more rapid and economical gains.

In all trials, less supplement was required per 100 lbs. of gain with the supplements containing the highest percentage of crude protein. However, about the same quantity of protein was required for all lots per 100 lbs. of gain. The use of pasture was of significant importance in reducing the cost per unit of gain. Pasture was also of supplementary value to the all-vegetable protein concentrate as indicated by the more economical gains.

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INCUBATED EGGS AND DEAD POULTRY AS HOG FEED

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SOME operators of small hatcheries dispose of their unhatched incubator eggs by feeding them to hogs. This practice not only provides a convenient method for the disposal of these wastes, but also returns a small salvage value to the hatcheryman.

Willman (1941) has reported that satisfactory gains were made by 50 pound hogs that were fed incubator eggs, yellow corn and salt. The hogs had free access to yellow corn and salt in self-feeders and were fed all the incubator eggs they would clean up three times a day.

This work was of particular interest because, from a theoretical point of view at least, there was some doubt concerning the value of raw egg white as a feedstuff. Several workers had shown that raw egg white was poorly digested by man, dogs, and rats. However, Willman, *et al.* (1942), showed that raw eggs were digested just as efficiently by the hog as cooked eggs. They concluded that there were two possible explanations of their results. Either the hog is unique in its ability to digest raw albumen or the holding of the eggs in the incubator so modifies the albumen that it is made digestible.

As far as can be learned, there are no reports on the use of dead poultry as hog feed. Swine are omnivorous and may offer a convenient method for the disposal of the dead poultry on poultry farms. There is some danger, however, of infection from feeding unsterilized dead poultry and incubator eggs, as swine are susceptible to certain avian diseases.

Experimental Results

In the fall of 1942, feeding trials were made at the Delaware Agricultural Experiment Station to study the value of these two wastes as hog feed. Fall-farrowed pigs averaging 50 pounds in weight were selected for the experiment, and fed for 105 days. Each group had the run of a one-third acre lot and an 8×8 colony house. One group was fed 3.6 pounds of yellow corn daily per 100 pounds of live weight and all the incubated eggs they would consume. Another group was fed the same amount of corn and all the dead poultry they would consume. The third group, which was used as the control, was fed 4.5 pounds of the following ration daily per 100 pounds of body weight: 100 pounds yellow shelled corn, 10 pounds of tankage (60 percent protein), 5 pounds of alfalfa meal, and 5 pounds of linseed oil meal. All three

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groups had free access to a mineral mixture containing 100 pounds of bone meal, 100 pounds of ground limestone and 50 pounds of salt.

Unfortunately the feeding trial had to be terminated after running 15 weeks so that yards could be prepared for sowing.

The average gain in weight and feed consumption with other information pertinent to the experiment is given in table 1.

TABLE 1. SUMMARY OF RESULTS OF A FEEDING TRIAL USING INCUBATED EGGS AND DEAD POULTRY AS HOG FEED

Items compared	Supplement fed		
	Tankage	Incubated eggs	Dead poultry
Number hogs in each group	10	3	5
Corn consumed per hog	313	255	199
Supplement fed, pounds	64	287	145
Average gain per hog	86	83	73
Pounds of purchased feed per pound of gain	4.38	3.07	2.73
Rate of gain per day	.89	.86	.76

The rates of gain per day are somewhat low, but the relative gains of the three groups are of interest. All groups went off feed twice during the feeding period.

Although it was planned to feed the incubated egg and dead poultry groups at the level of 3.6 pounds of corn daily per 100 pounds of body weight this level was not reached on the basis of the whole feeding period. In the beginning, the hogs had to be fed corn at a lower level in order to get them to eat the wastes readily and both times that the groups went off feed the amount of corn fed had to be cut back. Once the hogs got used to the eggs and dead poultry, however, they ate them with a relish and were always eager for the next feeding.

If the differences in amount of gain are not considered, 100 pounds of incubator eggs replaced 20.2 pounds of shelled corn, 10.9 pounds of tankage, 5.4 pounds of alfalfa meal, and 5.4 pounds of linseed oil meal while 100 pounds of dead poultry replaced 78 pounds of shelled corn, 21.4 pounds of tankage, 10.8 pounds of linseed oil meal and 10.8 pounds of alfalfa meal.

Conclusions

Incubated eggs from the hatchery and dead poultry on poultry farms may conveniently be disposed of by feeding to swine. The results of this feeding trial indicate that hogs fed on shelled corn, a mineral mixture, and either

dead poultry or incubated eggs do not gain as rapidly as hogs fed a conventional diet. Purchased feed required per pound of gain is low, however, and a small salvage value is realized for the wastes fed.

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AN APPRAISAL OF FACTORS AFFECTING PROLIFICACY IN SWINE[1]

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THERE is great variability in the size of litters of swine produced by females of the same age and breeding, and between litters from the same female. If a portion of this variability could be accounted for in terms of measurable factors related to prolificacy, and if the relative importance of those factors could be established, selection for fertility in swine should be more effective.

If progress toward increased litter size is to be made, potential productivity must be evaluated before the gilts are saved as breeders. Sows that survive the rigorous selection following their first farrowing become such a small and select group that they contribute but a very small amount to the total hog breeding population. The average lifetime production per sow in the Corn Belt is less than two litters, so in the cases where selection is based on production, the breeding females are selected on the basis of their dam's first litter.

Many workers have recognized the increase in litter size with age of sow at successive farrowings. Ellinger (1921), Johansson (1929), Krizenecky (1935), and Olbrycht (1943) have suggested a possible progressive increase in litter size with an increase in age at the time of first farrowing. Olbrycht found that sows at 12 months produced an average of 1.07 pigs less than those farrowing their first litters at 17 months and Johansson found the number of pigs in the first litter increased with the age of the sow to about 16 months.

During the past 25 years several state experiment stations as well as the U. S. D. A. have studied the effects of inbreeding on swine. The effect of inbreeding on fertility as measured in these studies varied considerably but in general litter size was found to decrease as inbreeding increased. Estimates of the reduction in pigs per litter varied from .5 to more than 1.3 for each ten percent increase in the coefficient of inbreeding.

Hetzer, Lambert, and Zeller (1940) reporting on the influence of inbreeding on litter size in Chester Whites concluded that inbreeding had no appar-

[1] Paper No. 2196, Scientific Journal Series of the Minnesota Agricultural Experiment Station in cooperation with the Regional Swine Breeding Laboratory, Ames, Iowa, Bureau of Animal Industry, United States Department of Agriculture. The results reported in this paper are part of a comprehensive study of the factors affecting productivity in swine which is being conducted by the University of Minnesota under the direction of Dr. L. M. Winters. The author is indebted to Dr. R. E. Comstock for guidance in the statistical procedures. This paper is condensed from a thesis submitted to the Graduate Faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of doctor of philosophy.

ent effect on the reproductive capacity of either boars or sows and their results suggest that it was primarily the inbreeding of the litter which adversely affected litter size. As sows of all ages contributed to the mean litter size, and as no correction was made for the effect of age differences between sows, even though they were recognized as existing, the effects of inbreeding are here confounded with the effects of these age differences.

In a study of crosses among inbred lines of guinea pigs, Wright (1922) decided that the inbreeding of the dam had an influence on litter size at birth but that the heredity of the young had no influence.

Although Wentworth and Aubel (1916) found no evidence of relationship between body characteristics and fertility in Poland China hogs, Phillips and Zeller (1943) working with the same breed found large type gilts reached sexual maturity earlier and at a more uniform age than those of the small type. From the work of Zeller (1940), Hetzer and Brier (1940), and Hankins (1940), it becomes apparent that in the study of type in Poland Chinas, the longer bodied females were also the heaviest at mating. Hetzer and Brier attributed a part of the differences in litter size between types to differences in sow weight or factors associated with it. When these workers removed differences between sows due to differences in weight they found highly significant differences between types of hogs. These differences in type are most evident in the body length of the carcasses at a uniform weight.

It seems logical that the well being of females, prior to ovulation, and during the periods of implantation and subsequent gestation has an effect on intra-uterine condition and prenatal death loss. The most practical measure of physical well being during this time is the increase in body weight. McKenzie (1928) found a marked relationship between daily gains made the four weeks after the sows settled and the number of the pigs farrowed. This general relationship held for gains made throughout gestation. Hogan and McKenzie (1927) found that sows gaining below the average of their group during the first month after breeding, produced litters below the average in size. These findings were substantiated by Zeller, Johnson, and Craft (1937), and Donald and Fleming (1938).

An attempt was made in this study to identify factors responsible for a portion of the non-genetic variance of litter size, and to determine the proportion of the total variance for which they were accountable.

Data and Methods of Analysis

The data used for this study were gathered in the Minnesota swine breeding project conducted in cooperation with the Regional Swine Breeding Laboratory of the U. S. Department of Agriculture. They were gathered over a period of seven years, 1937 to 1943, inclusive, and include farrowing

records on 14 inbred lines and a group of miscellaneous line crosses. Twelve of the lines contributing to these data were developed within the Poland China breed, one line was developed from a Landrace-Tamworth crossbred foundation (Winters *et al.*, 1943), while the other line is being developed from matings between two inbred Poland China lines and the Yorkshire breed.

The records were gathered by the swine herdsman at each of the stations and were reported on the standard preweaning and postweaning sheets used in the Regional Laboratory. Data from the preweaning sheets consisted of the weight of the female at breeding, weight at 112 days after the last service, the total number of pigs farrowed, and the number of pigs farrowed alive, while the postweaning sheets gave the weights and body measurements of the females as they came out of the feedlot, at approximately 200 pounds.

The herds at all five of the stations were under as nearly uniform conditions of management as it was possible to provide. All animals were self fed the same ration until after reaching market weight. Breeding gilts were kept separate from sows, and all pregnant females were put into rather high condition previous to farrowing. Each female was hand mated.

A large portion of the selection for fertility in these lines was made at weaning time. Since more litters were farrowed within the lines than could be fed out, only litters with the greatest numbers of pigs went on test. Breeding stock was selected from these test lots.

The data were grouped so that all of the gilts in a group were farrowed in the same season at the same station; therefore, they were raised under as nearly uniform environmental conditions as was practicably possible. All of the gilts in a group were from a single line and farrowed their first litters during the same season. The analyses of variance and covariance were made between and within these groups. This grouping made possible elimination of environmental variance associated with years and seasons. The regression coefficients were determined from the within group component, both for individual lines and the average of all lines, using the methods outlined by Fisher (1938), and Snedecor (1940). Tests to determine differences between the regressions for the different lines were insignificant so the information from all of the lines was pooled in further study of the regression relationships.

There appear to be differences in litter size between the lines but these apparent line differences are confounded with year and station effects which defy interpretation. Since this study is an attempt to determine the factors affecting selection for fertility and the relative effects of these factors, differences between the lines in any of the measures studied were of no concern,

so long as there is no differential response in litter size to the various measures.

Since data were not available in every criteria of measurement for every female, and since the different phases of this problem required different groupings, two analyses were made.

The Effect of Age and Inbreeding

The first phase of this study was made to determine the effects of age of dam, the inbreeding of dam, and inbreeding of the litter on the size of the first litter. The records of 749 gilts, farrowing for the first time at approximately 1 year of age, included the age of the dam in months, the coefficients of inbreeding for both the dam and her litter, calculated according to the method of Wright (1922*a*), and the total number of pigs farrowed as well as the number of pigs farrowed alive. A summary of the mean values of these measures, together with the percentage of stillborn, by lines, is given in table 1.

TABLE 1. MEAN VALUES FOR AGE, PERCENTAGE OF INBREEDING, AND PIGS FARROWED BY LINES

Line and station	No.	Age, mos. *a*	Inbreeding of dam, % *b*	Inbreeding of litter, % *c*	Farrowed		Percentage stillborn
					Alive *d*	Total d_1	
Minn. #1 NC	194	12.1	13.8	19.7	8.7	9.3	6.1
BS NW	69	12.3	23.0	28.6	6.3	6.7	5.6
ML	71	11.7	18.8	26.4	5.9	6.3	6.3
#2	19	11.8	00.0	19.6	9.1	9.6	5.5
BS WC	39	12.9	17.1	26.8	7.9	8.0	1.6
WL	49	13.2	17.0	25.2	7.2	7.4	1.4
V	68	12.2	17.0	24.6	8.0	8.1	1.8
C_1 Cent.	46	12.0	8.0	18.7	6.2	6.4	9.6
C_2	37	11.4	16.7	23.1	5.5	5.8	4.7
C_3	9	12.4	00.0	25.6	7.3	7.7	4.4
M SE	55	12.1	66.3	69.2	5.7	5.7	1.3*
C	18	11.8	13.6	27.4	5.5	5.8	4.8
G	16	11.7	11.1	19.5	6.4	6.4	1.0*
MC	17	11.5	7.7	15.7	5.5	7.2	23.8
Misc. crossed lines	42	12.4	22.9	7.5	6.8	6.8	7.1*
Total	749	12.2	19.2	25.5	7.2	7.5	3.5

* Apparent inconsistencies owing to rounding of numbers.

As several workers have shown an increase in the number of pigs farrowed in successive litters with the increasing age of the dam, a study was made of the effect of age of dam on size of the first litter. The age of all females was calculated to the nearest month. Ages at first farrow ranged from 9 to 16 months, with most individuals farrowing when 11 to 13 months old. Linear and quadratic regressions of litter size on age were calculated and tested for significance for both pigs farrowed alive and total pigs farrowed. The linear and quadratic regressions were highly significant in both cases and in each case the quadratic regression gave a significantly better fit to the data then did the linear regression.

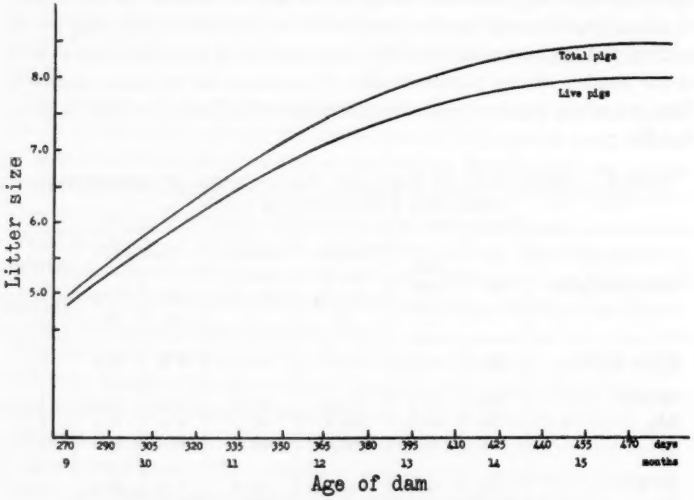

Figure 1. Curve of Litter Size on Age at First Litter from Quadratic Regression.

The quadratic regression line (figure 1) shows that litter size increases with the age of the dam, but that the rate of increase becomes less as age increases. According to these data there is no further increase after 15 months of age. This agrees with Johansson's (1929) results. He found the number of pigs in the first litter increased with the age of the sow to about 16 months. The effect of age on litter size is much more severe in the months before 12 than in those after that age. Differences could not be demonstrated between the regressions of total and live pigs farrowed.

The simple and partial regression coefficients determined for both the number of pigs farrowed alive and total pigs farrowed, on the age of the

dams, inbreeding of the dams, and inbreeding of the litters are presented in table 2.

TABLE 2. WITHIN GROUP SIMPLE AND PARTIAL REGRESSIONS OF PIGS FARROWED ALIVE (d), AND TOTAL PIGS FARROWED (d_1) ON AGE OF DAM (a), PERCENT INBREEDING OF DAMS (b), AND PERCENT INBREEDING OF LITTERS (c)

Simple regressions		Partial regressions		Partial regressions		Partial regressions	
da	.367**	$da \cdot b$	.548**	$da \cdot c$	.366**	$da \cdot bc$	.548**
d_1a	.415**	$d_1a \cdot b$	.535**	$d_1a \cdot c$	.415**	$d_1a \cdot bc$	.609**
db	−.023	$db \cdot a$	−.059**	$db \cdot c$	−.014	$db \cdot ac$	−.060**
d_1b	−.016	$d_1b \cdot a$	−.055**	$d_1b \cdot c$	−.013	$d_1b \cdot ac$	−.063**
dc	−.029	$dc \cdot a$	−.029	$dc \cdot b$	−.022	$dc \cdot ab$	.001
d_1c	.015	$d_1c \cdot a$	−.015	$d_1c \cdot b$	−.009	$d_1c \cdot ab$	.017

** Highly significant.

From these regressions it is evident, contrary to the findings of Hetzer *et al.* (1940), that in this case the inbreeding of the dam has a greater effect on prolificacy than the inbreeding of the litter and that the inbreeding of the litter has little or no adverse effect on litter size if the age and inbreeding of the dams are the same.

Age of the dam and the inbreeding of both the dam and her litter are all factors which may affect litter size. Estimates were made of the variation contributed by these factors by determining the ratio of the sum of squares due to regression, corrected for the effects of each measure, with the uncorrected sum of squares for litter size.* In the case of both the analyses of the pigs farrowed alive and total pigs farrowed, the total reduction in the sum of squares amounted to 3½ percent. Age alone accounted for about 3 percent of this total, and the inbreeding of the dam accounted for most of the remainder. The inbreeding of the litter contributed less than 0.1 percent to the sum of squares for litter size in these data. This indicates that the age of the gilt is of much greater importance than her inbreeding in selection for fertility.

The Effects of Weight at Breeding, Gains During Pregnancy, and Length of Body

This analysis was undertaken to determine the extent to which number of pigs farrowed alive in first litters is associated with differences in the age of

* (The sum of squares due to regression is the sum of the products of the partial regression coefficients and the appropriate sum of products.)

the dam, her weight at the time of breeding, the gains made during pregnancy, and body length. Age was included in this study because of its association with some of the other measures.

This analysis was based on the farrowing records of 508 of the gilts included in the previous study on which more extensive data were available. These females were grouped according to lines, seasons, and year of farrow. The data gathered included the age of the dam in months, her weight at breeding, the increase in weight to 112 days after breeding, body length and weight at the time the gilt was scored out of the test lot, and the number of pigs farrowed alive in her first litter. Body weight at the time of scoring was not considered to have an effect on fertility, but since there was some variation in the weight at which body length was measured, it was considered that this measurement should be corrected to a constant weight before attempting to interpret its effect on fertility. Body length was measured in inches, with wooden calipers, from a point directly between the ears to the point at which the tail emerges. The mean values of these measures are summarized by lines in table 3.

TABLE 3. MEAN VALUE FOR AGE OF DAM (*a*), PIGS FARROWED ALIVE (*d*), WEIGHT AT BREEDING (*f*), GAIN DURING PREGNANCY (*g*), WEIGHT (*h*) AND BODY LENGTH (*i*) AT MEASURING, BY LINES

Line and station		No.	*a*	*d*	*f*	*g*	*h*	*i*
			months	*pigs*	*lbs.*	*lbs.*	*lbs.*	*inches*
Minn. #1	NC	163	12.0	8.8	290.7	171.2	210.3	42.2
BS	NW	46	12.2	6.0	291.4	188.0	203.6	40.7
ML		57	12.0	6.0	283.3	189.4	212.4	40.8
Minn. #2		13	11.8	9.3	305.8	159.6	211.5	40.6
BS	WC	34	13.0	7.9	356.9	163.8	214.1	40.7
WL		36	12.9	7.1	346.1	160.8	213.0	40.3
V		41	12.5	8.2	331.1	155.0	211.5	40.2
C_1	Cent.	21	11.6	5.8	291.3	146.6	224.8	41.2
C_2		21	11.1	5.1	274.3	163.6	196.4	39.5
C_3		4	12.3	9.3	391.8	137.0	204.8	39.7
M	SE	34	12.0	5.6	296.5	124.6	205.7	40.6
C		1	11.0	6.0	265.0	151.0	223.0	41.4
Misc.		37	12.4	6.7	324.2	153.7	205.0	39.8
Total		508	12.2	7.4	304.9	165.9	209.8	41.0

In order to determine the importance of each of these measurements, both independently and collectively, simple and partial regression coefficients

were determined within lines and years for the number of live pigs farrowed on each of the 5 variables. These values are presented in table 4. The linear regressions of litter size on age, weight at breeding, and gain, are all highly significant.

TABLE 4. SIMPLE AND PARTIAL REGRESSION COEFFICIENTS DETERMINED FROM COVARIANCE WITHIN GROUPS FOR LIVE PIGS FARROWED (*d*) ON AGE OF DAM (*a*), WEIGHT AT BREEDING (*f*), GAIN DURING PREGNANCY (*g*), WEIGHT (*h*) AND BODY LENGTH (*i*) AT MEASURING, BY LINES

Simple and partial regressions		Partial regressions		Partial regressions	
$da \cdot$	.517**	$da \cdot fg$	.244	$da \cdot fhi$	.244*
$da \cdot f$	.517	$da \cdot hi$	.529	$da \cdot ghi$	.480
$da \cdot g$	.468			$da \cdot fghi$	.236
$df \cdot$	.012**	$df \cdot ag$	.008	$df \cdot ahi$	.008*
$df \cdot a$	.008	$df \cdot hi$	.013	$df \cdot ghi$	.013
$df \cdot g$	.012			$df \cdot aghi$	.009*
$dg \cdot$	.013**	$dg \cdot af$	.011	$dg \cdot ahi$	.011
$dg \cdot a$	.011	$dg \cdot hi$	.012	$dg \cdot fhi$	.011
$dg \cdot f$	.012			$dg \cdot afhi$	.012*
$dh \cdot$	−.003	$dh \cdot fi$	−.021	$dh \cdot agi$	−.002
$dh \cdot i$	−.011	$dh \cdot gi$	−.012	$dh \cdot fgi$	−.007
$dh \cdot ai$	−.011	$dh \cdot afi$	−.003	$dh \cdot afgi$	−.005
$di \cdot$	.123	$di \cdot fh$	.150	$di \cdot agh$	.190
$di \cdot h$	.169	$di \cdot gh$	.162	$di \cdot fgh$	.148
$di \cdot ah$	.194	$di \cdot afh$	.176	$di \cdot afgh$	.164

* Significant.
** Highly significant.

The size of the regression of litter size on body length was increased by holding weight constant although this partial regression still is not significant. Even though a significant regression cannot be demonstrated from these data, the trend is positive. The error in making the measurements for body length of live hogs contributes a great deal to the variance of this measure and tends to conceal any advantage this measure may have in making selections. The standard deviation for body length in these data is 1.0475 inches, while Fenske (1939) found a standard deviation of 0.753 inches in repeated measurements on the same animal.

The effects of the correlations between age at farrowing and both weight at the time of breeding, and gain during pregnancy are reflected in the partial regressions including these variables. The correlation between

age and weight at mating is +0.595, while that between age and gain is +0.135. The effects of each of these variables on total variance are not additive, however, in this case. The total effect can be obtained only from a multiple regression analysis.

The percentage reduction in variance for litter size for various measures is shown in table 5. Mathematical adjustment for all of the variates resulted in a reduction of 6.2 percent in this variance. The effects of age and weight at mating on the size of litter farrowed are about equal, as age alone reduced the variance for litter size by 3.2 percent and weight alone by 3.3 percent. These two measures together account for 4 percent of the variance.

TABLE 5. PERCENTAGE REDUCTION IN THE SUM OF SQUARES FOR LIVE PIGS FARROWED IN FIRST LITTERS, CORRECTED FOR THE EFFECTS OF AGE AND BODY MEASUREMENTS OF THE DAM

Measurements corrected for	Percentage reduction	Measurements corrected for	Percentage reduction
Age alone	3.2	Gain alone	2.2
Age and weight	4.0	Length (wt. constant)	.04
Weight alone	3.3	Age and gain	4.7
Weight and gain	5.3	Age and length	3.8
Age, weight, gain and length (wt.)	6.2	Age, weight, and length (wt. constant)	4.2

The actual reduction in the sum of squares for litter size obtained by mathematically adjusting for all of the variables, except weight at mating, is significant, while the reduction omitting age alone is not significantly different from zero. This indicates that weight at the time of mating has greater influence on litter size than age. Differences in age, and differences in weight, each reflect differences in sexual maturity as revealed through the number of pigs in first litters. Early sexual maturity apparently is associated more closely with growth than with age. In making selections, the use of both weight and age together more accurately predict prolificacy than either of these measures used alone.

The highly significant reduction in the sum of squares when the variable, gain during pregnancy, is removed indicates that there is less variation in litter size among gilts making equal gains, and that gilts gaining the greatest amount during the gestation period, on the average, may be expected to farrow the largest litters.

Since length of body at a constant weight reduces the sum of squares for litter size so very little, about 0.04 percent as shown in table 5, it appears that age and weight at breeding are the important variables to correct for

when making selections. These factors vary independently of the genetic ability of the gilts to reproduce.

Gilts bred when older and heavier than the average should be expected to farrow larger litters than the average, but the total variance in litter size would be reduced very little by breeding all of the gilts at the same age and weight. Selection of gilts farrowed within the same season on the basis of weight at breeding alone should be almost as efficient as the use of age and weight together, and selection by weight alone should be more efficient than age alone.

Summary and Conclusions

The effects of age and inbreeding of the dam, and inbreeding of the litter on litter size were studied from intra line, intra year simple and partial regressions calculated from the records of 749 inbred Poland China and Minnesota No. 1 gilts farrowing for the first time at approximately one year. The relative effects of age, weight at breeding, gains made during pregnancy, and body length were determined from the data on 508 of the gilts.

Litter size increased with an increase in the age of the dam at farrowing. The effect of age was much greater during the period prior to 12 months than later. Gilts farrowing at 320 days averaged 1 pig less, and those farrowing at 410 days about one half pig more than those at one year.

Litter size decreased with an increase in the inbreeding of the dam but apparently was unaffected by the inbreeding of the litter. An increase of ten percent in the inbreeding of dams of the same age resulted in a decrease of about 0.6 pigs per litter.

The heavier gilts at breeding, on the average, farrowed larger litters. The correlation between age and weight at mating was approximately .60.

Body length may be associated with prolificacy but errors in measurement conceal its value for use in selection.

On the average, gilts making the greatest gains during pregnancy farrow the largest litters but variations in gain may be an effect rather than a cause of variation in litter size.

Age and weight at mating together account for four percent of the variance in size of first litters and together they provide the most reliable criteria for use in selection for fertility.

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THE FACTOR IN EGG YOLK AFFECTING THE RESISTANCE, STORAGE POTENTIALITIES, AND FERTILIZING CAPACITY OF MAMMALIAN SPERMATOZOA

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PHILLIPS and Lardy (1940) described an egg yolk-phosphate buffer mixture which prolonged the storage and maintained the fertilizing capacity of bull spermatozoa for periods exceeding that of spermatozoa in undiluted semen. Subsequent investigations by Easley, Mayer and Bogart (1942), Lasley, Easley and Bogart (1942), Salisbury, Fuller and Willett (1941), Lasley and Bogart (1943, 1944, 1944*a*), Lasley and Mayer (1944) have substantiated the original results of Phillips and Lardy. That egg yolk might produce its effects on spermatozoa by increasing their resistance to adverse environmental conditions was first indicated by Lasley, Easley and Bogart (1942) who showed that bull spermatozoa varied in their inherent ability to resist environmental changes, that the number of resistant cells may, in some cases, be zero, and, that the addition of egg yolk in phosphate buffer could restore their ability to resist the conditions of storage.

Following these two significant contributions to spermatozoan physiology a series of reports from the Missouri Agricultural Experiment Station furnished additional information regarding the resistance of spermatozoa, the effect of egg yolk and the correlation of resistance with storage and fertilizing capacity. It was shown (Lasley and Mayer, 1944; Lasley and Bogart, 1944, 1944*a*) that epididymal spermatozoa of the bull and boar are highly resistant, but lose their inherent ability to resist adverse environmental conditions as they progress through the male genital tract. Of noteworthy significance was the finding that egg yolk had little or no effect upon epididymal spermatozoa. Further, it was shown that ejaculated spermatozoa, in some instances, possess little or no resistance, yet in every case have the capacity to acquire resistance from the yolk-phosphate diluter. As our knowledge of this cellular resistance increases, more significance may be assigned to the fact that the total number of resistant cells in egg yolk diluter (Easley, Mayer, Bogart, 1942; Lasley and Mayer, 1944; Lasley and Bogart, 1944, 1944*a*) was found to be quite constant irrespective of the number of cells inherently resistant before adding the yolk diluter. Since fluids from the epididymis and the male accessory glands have no influence on the inherent cellular resistance (Lasley and Bogart, 1944, 1944*a*), it has been concluded

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that this resistance is a function of the cell and that variations in its magnitude are the result of cellular changes.

In contrast, the resistance which ejaculated spermatozoa acquire upon the addition of yolk-phosphate diluter must evidently be dependent upon the presence of some factor (or factors) in the egg yolk which initiates or produces changes in the cell resulting in an increased resistance to unfavorable environmental conditions. Two problems are presented by this type of spermatozoan resistance; one of which is concerned with the nature of the egg yolk factor and the other with the mechanism whereby the factor influences cellular resistance.

The present paper is a preliminary report of our investigations designed to gain information regarding the identity of the resistance factor of egg yolk.

Materials and Methods

In their 1940 report Phillips and Lardy stated that egg yolk alone was less effective than the buffered egg yolk which suggested that phosphate buffers might provide good media for the study of the yolk factor.

In order to reduce experimental variables to a minimum an isosmotic mixture of Na_2HPO_4 and NaH_2PO_4 having a pH of 7.35 to 7.4 was utilized. One liter quantities of M/8 solutions of each of the sodium phosphates were prepared. These will remain unchanged for as long as two months in the refrigerator. Fresh buffer of pH 7.35–7.4 was prepared for each experiment by adding 1 volume of M/8 Na_2HPO_4 to 4 volumes of M/8 NaH_2PO_4, as mold formation occurs in this buffer within 2 or 3 days even at refrigerator temperatures.

Absolute ethyl alcohol was always used for the extraction or washings of precipitates wherever the use of alcohol is indicated in this report. The absolute ether was prepared from C. P. ethyl ether by thoroughly washing the ether with a 1 percent ferrous sulfate solution followed by sufficient washings with distilled water to remove the sulfate. The latter washings were deemed completed when the wash water no longer gave a precipitate with $BaCl_2$. The washed ether was distilled over $CaCl_2$ to remove water.

The relative resistance of the spermatozoa was measured by subjecting ing 0.5 ml. of undiluted semen or of semen diluted 1:3 with phosphate buffer solutions containing egg yolk or egg yolk fractions to the cold shook technique as described in detail by Lasley and Bogart (1943) and Lasley and Mayer (1944). The resistance was expressed in percentage of cells surviving this severe test; the surviving or live cells being determined by the staining method of Lasley, Easley and McKenzie (1942). For comparative purposes the percentage of live spermatozoa in the freshly collected undiluted semen

and in the semen immediately after addition of a diluter was obtained. The accuracy of the method for determining the number of live cells in a sample was presented by Lasley, Easley and McKenzie (1942). Motility was also recorded for all specimens immediately following collection, after dilution, and after application of the cold shock technique.

Experimental Results

Supernatant of Egg Yolk-Buffer Mixture

Before any progress in the isolation of the resistance factor of egg yolk could be made, it was necessary to determine the form in which the factor occurred in the egg yolk-buffer mixture, i.e. as an insoluble precipitate, as a colloidal suspension or as a completely soluble substance. In an endeavor to answer this problem one volume of egg yolk was diluted with 4 volumes of the isosmotic Na_2HPO_4-NaH_2PO_4 buffer at pH 7.4 and at pH 8.0. It was found that a clearer supernatant could be obtained at the higher pH. After centrifugation the clear amber supernatant was mixed with bull semen in the ratio of 1 volume semen to 3 volumes supernatant. The clear supernatant was found to be as effective in protecting the cells against the adverse conditions of the cold shock technique as the original uncentrifuged egg yolk-buffer mixture, indicating that the active resistance factor was soluble in the buffers both at pH 7.4 and at pH 8.0. Since the supernatant contained lipid droplets which interfered during microscopical examination, a further step in purification with a lipid solvent was in order.

Buffer Extract of Acetone Precipitate

The proteins and other acetone insoluble substances were precipitated from egg yolk by the addition of 4 volumes of acetone and the precipitate washed with acetone to remove the greater proportion of the lipids. The yellowish precipitate was dried in vacuo to remove acetone, then extracted with phosphate buffer (pH 7.4) followed by filtration or centrifugation of the buffer extract to remove insoluble material. The clear colorless supernatant of the buffer extract again showed activity similar to that of the original egg yolk.

Effect of Alcohol and Ether Extraction

Although the active substance was now in a water-clear, fat-free solution, its activity had not increased above that exhibited by the original egg yolk-buffer mixtures. As a further step in purification the dried acetone precipitate was ectracted in a Soxhlet extractor with hot absolute alcohol for 8 hours, the alcohol removed, and the precipitate further extracted for 4 hours with peroxide-free absolute ether. Interestingly, the ether removed but a

trace of lipid material; apparently only that in solution in the alcohol remaining in the precipitate. The ether extraction has been retained as a step in the isolation of the egg yolk factor to remove the last traces of alcohol and alcohol-soluble substances. The white fluffy lipid-free precipitate was dried in vacuo and then extracted with phosphate buffer as before. This clear buffer extract protected 68.5 percent of the spermatozoa during the cold shock test as compared to a protection of but 51.5 percent by the original egg yolk-buffer mixture—an increase of 33 percent in the number of cells surviving the test. These results are presented in table 1 and are compared with percentage survival of spermatozoa in semen diluted with phosphate buffer alone. Apparently no protection was afforded by the buffer solution without the factor as only 30 percent of the cells survived the test (Lasley and Bogart, 1943; Lasley and Mayer, 1944).

TABLE 1. EFFECT OF EGG YOLK FRACTIONS IN PHOSPHATE BUFFER (pH 7.4) ON RESISTANCE OF BULL SPERMATOZOA

Fraction	Characteristics of fraction[1]	Effect on semen diluted 1:3 with factor-buffer solution		
		Immediate motility**	Motility after 0° C. for 10 minutes	Percent alive after 0° C. for 10 minutes
Fy D	Buffer soluble, insoluble in acetone, alcohol, ether	5	5	68.5
Normal* semen	Diluted with phosphate buffer 1:3	5	2	30.0*
Egg yolk in buffer	—	5	5	51.5

* Semen in buffer 1:3 had original motility of 5 and 87 percent were alive.

** Motility rated by numerals from 1 to 5; the 1 denoting minimum and the 5 maximum motility.

[1] Discarded fractions of egg yolk were mostly insoluble in buffer and either entirely killed sperm or lowered percentage alive after cold shock to 10 percent or less.

A series of experiments at this stage showed that two factors were acting upon the spermatozoa—the resistance factor and an alcohol-soluble toxic substance which in most cases killed the spermatozoa immediately upon its addition to the media. In table 2 are presented data which show the effect of removing the alcohol-soluble factor. Approximately a 92 percent increase in the number of cells surviving the cold shock test resulted from the removal of the toxic factor by extraction with alcohol. It will also be noted that addition of the toxic factor decreased the number of surviving cells below that occurring in buffer alone.

TABLE 2. EFFECT OF REMOVAL OF TOXIC FRACTIONS ON EGG YOLK FACTOR

Fraction number	Description of fraction	Results after 0° C. for 10 minutes on ram semen diluted 1.4 with buffer extract of fraction
I	Acetone precipitate of fresh egg yolk	12% alive
II	Acetone ppt. extracted with alcohol and ether (extracted residue)	23% alive
III	Alcohol soluble portion of acetone ppt.	6.8% alive
Phosphate buffer	—	10% alive

Recovery on an Active Fraction From the Buffer Extract

Since removal of the moisture and the acetone, alcohol, and ether soluble fractions left a residue comprising but 18 percent of the original egg yolk (table 3), it was evident that decided progress towards purification of the active fraction had been made and methods for the recovery of this fraction from the buffer extracts of the above residue were sought. Many attempts to isolate the active fraction in the buffer solution by formation of insoluble salts or complexes were made. Some were successful, but each produced a product requiring the removal of contaminating substances used in the isolation.

Precipitation of the active fraction by the addition of acid proved to be the most desirable method. The active fraction was found to precipitate from the buffer solution at a pH of 2 to 3 as a white, powdery substance representing approximately 1.5 percent of the original egg yolk or 8 percent of the moisture free, acetone, alcohol and ether extracted residue. Re-solution of the acid precipitated fraction in buffer (1 mg. per ml.) gave a solution possessing activity identical with that of the buffer extract from which it was recovered. The step by step procedure for the isolation of the active fraction of egg yolk is presented in figure 1.

The percentages of the various fractions of egg yolk separated by the procedure given in figure 1 are shown in table 3. The degree of purification produced by the organic solvent treatment of the egg yolk is here illustrated. It will be noted that these solvents (acetone, alcohol, and ether) remove 66 percent of the dried egg yolk leaving a residue of but 34 percent.

Chemical Characteristics of the Active Factor

Although the active factor has not as yet been isolated, its concentration

in an active fraction by a definite chemical procedure does give considerable insight into some of its characteristics. The method of isolation (figure 1 indicates that the active factor is insoluble in acetone, alcohol and ether, soluble in water as the sodium salt and insoluble as the acid. Other tests

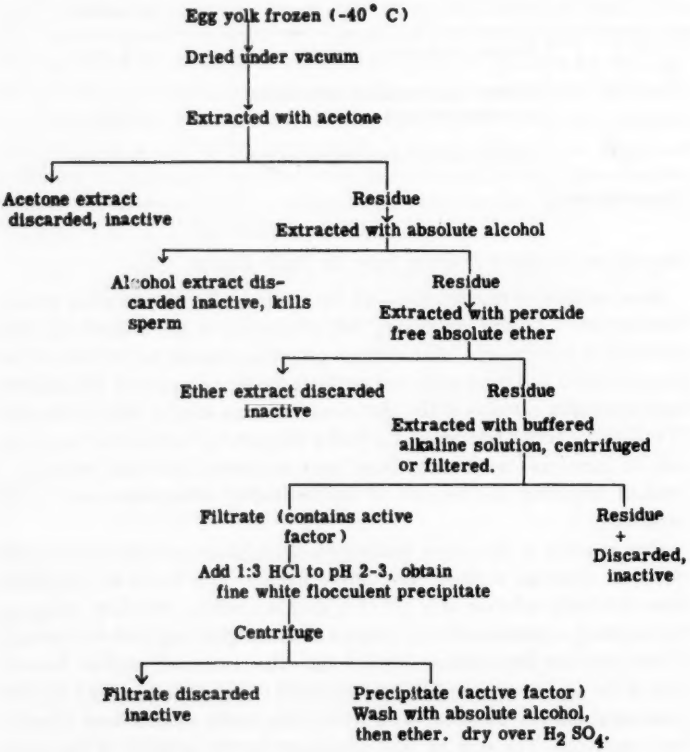
Egg yolk frozen (-40° C)
Dried under vacuum
Extracted with acetone
Acetone extract discarded, inactive
Residue
Extracted with absolute alcohol
Alcohol extract discarded inactive, kills sperm
Residue
Extracted with peroxide free absolute ether
Ether extract discarded inactive
Residue
Extracted with buffered alkaline solution, centrifuged or filtered.
Filtrate (contains active factor)
Residue + Discarded, inactive
Add 1:3 HCl to pH 2-3, obtain fine white flocculent precipitate
Centrifuge
Filtrate discarded inactive
Precipitate (active factor) Wash with absolute alcohol, then ether. dry over H_2SO_4.

Figure 1. Method of isolation of resistance factor from egg yolk.

have shown that the active fraction (and with it the factor) is soluble in benzene. A suspension of the active fraction in water has a pH of 3.5 suggestive of the acid character of the active fraction and possibly of the active factor.

Discussion

On the basis of previous reports by Missouri investigators, of results reported in this paper, and of unpublished data, the varied behavior of

ejaculated and epididymal spermatozoa of the boar, bull and ram towards adverse environmental conditions has been attributed to some cellular change of a morphological, physiological or chemical origin which affects their resistance. Two types of resistance have been recognized, viz. inherent and

TABLE 3. PERCENTAGES OF VARIOUS FRACTIONS OF EGG YOLK SEPARATED BY THE PROCEDURE GIVEN IN FIGURE 1

Original egg yolk		Dried egg yolk	
Fraction	Percent	Fraction	Percent
Moisture	46.8	—	—
Acetone soluble	25.1	Acetone soluble	47.3
Alcohol and ether soluble	10.1	Alcohol and ether soluble	18.9
Residue	18.1	Residue	33.9
Active fraction	1.5	Active fraction	5.0

acquired. Over 90 percent of epididymal spermatozoa are inherently resistant (Lasley and Bogart, 1943, 1944, 1944*a*; Lasley and Mayer, 1944) but lose their resistance as they progress through the male genital tract until at ejaculation none or only a small proportion retain the inherent capacity to resist environmental changes.

However, some 50 to 70 percent of ejaculated bull spermatozoa are resistant in the presence of egg yolk irrespective of the number possessing inherent resistance at ejaculation. It has been shown that some factor in egg yolk enables a varying proportion of ejaculated spermatozoa to acquire resistance. Since but a fraction of the spermatozoa alive at the time of ejaculation are capable of becoming resistant in the presence of egg yolk or of egg yolk factor, the acquired resistance is dependent upon two requirements, viz. some condition in the spermatozoa of a physiological or morphological nature which enables the cell to respond to the egg yolk factor and secondly, viz. some condition in the spermozoa of a physiological or morphological na ture which enables the cell to respond to the egg yolk factor and secondly, the presence of some factor in egg yolk which is necessary before the cell can become resistant.

Individual animals within a species and the different species of farm animals vary in the number of cells in semen which are inherently resistant at ejaculation and thusly, the semens vary in storage capacity in the absence of the resistance factor of egg yolk. In the bull addition of the resistance factor has, in our experience, produced a nearly constant number of cells resistant after the application of the cold shock technique—between 50–55 percent with egg yolk and 65–70 percent with the purified factor (table 1 and previ-

ous publications by Lasley and Bogart and by Lasley and Mayer). Table 2 shows typical results obtained by Missouri investigators with boar and ram semen. Although spermatozoan resistance in the epididymis of this animal is about 90 percent, the precentage of cells resistant in freshly ejaculated semen is less than 15 percent. Interestingly, the addition of egg yolk seldom increases the number of resistant cells above 30 percent (tables 1 and 2, this paper; Lasley and Bogart, 1943, 1944, 1944*a*; Mayer and Lasley, 1944). The authors cite the above data as a basis for their opinion that the egg yolk-buffer diluter has not proved as successful in the storage of ram and boar semen as compared with its successful use in the storage of bull semen because fewer cells in boar and ram semen are capable of acquiring resistance in the presence of the egg yolk resistance factor.

The active fraction of egg yolk which is described in the present report should be of practical value in the examination and storage of semen for subsequent insemination since the water clear buffer solution of the fraction should facilitate microscopical and other techniques of semen evaluation while the storage and fertilizing capacity of the specimen should exceed that obtained in an egg yolk-buffer mixture.

The actual factor responsible for the increased resistance of spermatozoa may comprise the bulk of the active fraction or the factor may be present in the active fraction in minute quantities. When we possess sufficient knowledge both of the inherent and of the acquired resistance of spermatozoa and can definitely identify the yolk resistance factor, it may be that decided progress will have been made toward the elucidation of the practical problems related to the storage and shipment of semen and to the preservation of its fertilizing capacity. This opinion is supported by the discovery of a high degree of correlation between spermatozoan resistance and the storage and fertilizing capacities of these cells (Lasley and Bogart, 1943; Lasley and Mayer, 1944; Lasley, 1944).

Summary

The division of spermatozoan resistance into two distinct types has been suggested and the relationship of each to problems of reproductive physiology has been discussed.

The procedure has been presented for the isolation of an active resistance factor from egg yolk which gives a water-clear solution in phosphate buffer and which has proved more effective than the original egg yolk-buffer mixture in increasing the resistance of spermatozoa to adverse conditions. It has been shown that the increased effectiveness may be the result of the removal of an alcohol-soluble fraction which is detrimental to spermatozoan viability.

Some of the chemical characteristics of the active fraction of egg yolk have

been described. It may be that the active factor in this fraction possesses similar characteristics.

Further, identification of the factor present in the active fraction and knowledge of the mechanism whereby it influences spermatozoan resistance should aid, it has been suggested, in the solution of some of the problems of reproductive physiology as well as making a definite contribution to our knowledge of general cellular physiology.

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LIVABILITY AND GLYCOLYSIS OF BOVINE SPERMATOZOA IN YOLK-CITRATE, INCUBATED EGGS OR CHICK-EMBRYO DILUTERS

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SINCE the first preliminary report by Frank, Smith and Eichhorn (1941) of the period over which bovine spermatozoa motility might be maintained in a chick-embryo diluter no detailed report on this subject has come to the attention of the authors. Nor have those workers published detailed descriptions of how their diluter was prepared. Later results, summarized by Mohler (1941, 1942) indicated that their chick-embryo diluter was of value in preserving motility of spermatozoa stored at 2° C., and up to 48 hours after collection the semen in this diluter was equally as efficient in maintaining fertility as the "egg-yolk diluting fluid."

Zelaya (1942) found diluters composed of the whole-fertile-egg incubated for 10 and 15 days at 102° F. were superior in maintaining motility to a yolk-phosphate diluent. On the other hand, a similar diluter made from eggs incubated 5 days was not superior in maintaining motility on storage.

Recently, Lichty (1943) reported on the conception rate from semen samples divided and used with a chick-embryo diluter and the yolk-phosphate diluent. While superior results for the chick-embryo diluter were reported, the number of services for each diluent were too few on which to base definite conclusions.

These reports have all been limited in scope, but as they have all suggested a superiority of a chick-embryo diluter over others in common use, it was considered desirable to study the matter further.

Therefore, an investigation was planned to determine the livability of bull spermatozoa both during incubation for 1 hour at 46.5° C., and for 10 days at a storage temperature of 5° C. in a whole-fertile-incubated egg diluter in comparison to the livability in the yolk-citrate diluent in common use here. In addition, it was believed that a study of glucose break-down to lactic acid, glycolysis, by the spermatozoa in these two diluters would serve as a check on the motility observations. Evidence is available which suggests that a major share of the energy for spermatozoa motility is obtained from glycolysis, Lardy and Phillips (1941), Mac Leod (1941).

Experimental Procedures

Two different experiments were conducted; one during August when 10 individual ejaculates were collected and used, and the other during the

following March when 11 ejaculates were used. The semen was collected and used almost immediately for incubation or for low-temperature storage studies. The experiments were factorially designed for statistical analysis. Each ejaculate of semen was diluted at the standard rate of 1 part of semen to 4 parts of each diluter studied. Observations of motility and determinations of pH were made before and after incubation at 46.5° C. for 1 hour, and before storage and after 2, 4, 6, 8, and 10 days' storage at 5° C. When the samples were to be stored they were cooled from body temperature to the storage temperature in steps of a 5° C. change each 20 minutes.

Aliquot samples of the diluted semen were taken before and after incubation or low temperature storage and immediately frozen for later analysis. Protein-free filtrates of the diluted semen were prepared by the method of Somogyi (1930). Total reducing substances were determined by the Horvath and Knehr (1941) adaptation to the photoelectric colorimeter of the Folin-Malmros micro blood sugar method. Incubation of each diluter, with and without semen in addition, with *Streptococcus fecalis*[1] bacteria, when properly treated before use almost specific fermenters of glucose, indicated that all of the reducing substances in the chick-embryo diluter were fermentable. In the yolk-citrate diluent about 20 to 30 mg. per 100 ml. of the total reducing substances was not fermentable. In spite of these facts no attempt has been made to correct the data for the yolk-citrate diluent to a fermentable sugar basis, instead the total reducing substances are reported and referred to as glucose in this study. Lactic acid was determined by the method of Barker and Summerson (1941).

The yolk-citrate diluter was prepared using 1 part of fresh egg yolk and 1 part of the sodium citrate buffer. The buffer solution was made of either 4.76 grams of 2 $Na_3C_6H_5O_7 \cdot 11H_2O$ added to 100 ml. of water distilled over glass, or 3.92 grams of $Na_3C_6H_5O_7 \cdot 2\ H_2O$ to 100 ml. of similarly distilled water. The buffer solution was autoclaved before use. The first chick-embryo diluter was made up of whole fertile eggs incubated 11 days in a forced-draft incubator held at 99.5° F. The entire egg contents were placed in a Waring blendor, thoroughly blended for 5 minutes and centrifuged for 20 minutes to one-half hour. Centrifugation threw down the heavier, larger portions of material which were discarded. About 70 percent of the egg contents were finally used. In the second experiment 10 whole fertile eggs incubated 9 days were used except that, in the case of four of these eggs, the outer albumen was mechanically removed before blending. These two diluters we have called chick-embryo I.

Also, in this last experiment a diluter made only of the chick embryo

[1] The authors are indebted to Professor I. C. Gunsalus, Laboratory of Bacteriology, Cornell University, for these cells.

itself was used. The individual live embryos were removed with a sterile forceps, blended and centrifuged as before. The blended material from which the coarser particles were removed by centrifugation was used for the last three ejaculates in the second experiment. This diluent has been labeled, chick-embryo II. The last three ejaculates in the second study were also diluted with yolk-citrate and with the whole-fertile-incubated-egg diluter (C.E.I).

The semen in the two experiments was of greatly differing quality, that in the first experiment being the poorest. However, the samples in the two experiments ran the gamut of semen quality. The spermatozoa counts ranged from 403,000 to 1,482,000 per mm.^{3}, the mean being 930,190 spermatozoa per mm.^{3}. The initial motility of the various samples ranged from 40 to 80 percent and the methylene blue reduction time in yolk-citrate from 5 minutes to 1 hour. Samples of this great variability were used largely to determine whether or not all samples would react the same to the different diluters.

Results

The first difference noted between the same semen samples in the two diluters compared, had to do with the rate at which these samples reduced methylene blue. The 21 ejaculates reduced methylene blue in yolk-citrate in a mean time of 23.0 minutes; the same semen samples in the chick-embryo I doing so in 10.9 minutes. The difference in reduction time was statistically significant and especially marked with the sample requiring the longest time to reduce the dye in the yolk-citrate. In check runs with the diluter alone it was found that the chick-embryo diluter would partially reduce the dye by itself in 1 hour at 46.5° C., but would not completely reduce the dye in 2 hours. Apparently the chick-embryo material supplied substrates or enzymes which speeded up hydrogen transfer, thus decreasing the time required to reduce the dye. However, as will be shown later, this increase in the rate of hydrogen transfer at 46.5° C. was not reflected by a similar rate of increase in glycolysis at the same temperature or during the low-temperature storage.

As the results were similar for both experiments the mean data of the comparison combining the two individual studies together are shown in table 1. This table gives the following data: the initial percent of motile spermatozoa, the initial rate of motility, the initial glucose level, the initial lactic acid level, the mean percent of motile spermatozoa, and the mean rate of motility after 1 hour incubation and after low-temperature storage for the several time-intervals. The data on glucose and lactic acid during incubation or storage are presented as loss and gain respectively.

An analysis of variance of the data shows that the percent of motile

spermatozoa in the chick-embryo diluter was maintained at a statistically significant higher level, both during incubation and in storage than was the case with spermatozoa in the yolk-citrate diluent. The greatest difference was noted for the semen samples of poorest quality. However, the mean difference in percent of motile spermatozoa in the diluents was not of great magnitude and, not of practical importance. The rate of movement of the individual spermatozoa (4.0 being the standard for the optimum speed of progressive movement) was not significantly different for the two diluters.

TABLE 1. COMPARISON OF SEMEN IN Y.C. AND C.E. I DILUTERS (COMBINED DATA OF TWO EXPERIMENTS)

Items compared	Initial	After 1 hour at 46.5° C.	After storage at 5° C. for				
			2 days	4 days	6 days	8 days	10 days
Motility %							
Y.C.	61.4	24.1	46.7	40.7	31.2	21.2	16.8
C.E. I	61.4	29.3	46.2	42.9	32.9	27.1	17.9
Rate of motility							
Y.C.	3.20	1.87	2.71	1.98	1.46	1.10	.80
C.E. I	3.20	2.05	2.43	1.95	1.40	1.19	.83
Initial glucose level and loss after storage, mg./100 ml.							
Y.C.	195.5	51.6	40.6	54.4	64.0	65.3	78.6
C.E. I	202.7	39.6	34.3	39.1	49.1	53.6	64.6
Initial lactic acid and gain during storage, mg./100 ml.							
Y.C.	22.4	37.2	31.3	43.7	50.9	57.3	53.3
C.E. I	84.0	38.5	38.6	42.5	61.2	57.9	60.8
Blanks							
(glucose level) mg./100 ml.							
Y.C.	104.6	100.4	105.3	108.1	109.0	94.2	84.6
C.E. I	109.2	102.9	105.5	110.2	108.75	114.4	121.5
(lactic acid level) mg./100 ml.							
Y.C.	9.6	10.2	8.8	7.8	5.1	7.9	12.3
C.E. I	74.2	67.2	69.4	70.3	75.8	77.1	76.5

The glucose loss during incubation, or storage, on the other hand was greatest for the spermatozoa samples in the yolk-citrate diluent. The difference between the diluters was highly significant. Conversely the greatest increase in lactic acid was observed with the chick-embryo diluter, both during incubation and long-time storage. Also, the highest proportion of the glucose-loss recovered as lactic acid gain was found for the chick-embryo I diluter. In fact, for low-temperature-storage at 2, 4, 6, and 8 days more lactic acid was produced than glucose was lost in the chick-embryo. This fact suggests that the spermatozoa in this diluent converted to lactic acid other nutrients than those determined as glucose in this study.

On the other hand, the data on the blank determinations of the diluters without added semen, but otherwise handled the same as the other samples, indicates that there may be some shifts of glucose-lactic acid relationships in the diluter during storage. These data suggest that a part, at least, of the difference between the diluters in percent recovery of initial glucose as lactic acid may be due to a slight decrease in the total reducing substances during storage in the yolk-citrate and an increase in such substances in the chick-embryo diluter during storage for 10 days.

However, this explanation does not account for the higher recovery of glucose as lactic acid during high-temperature incubation in the chick-embryo. This fact suggests that some mechanism, itself, or as a result of an interaction with semen, has a tendency to maintain the spermatozoa in the

TABLE 2. MEAN DATA ON THREE SEMEN EJACULATES STORED IN THREE DILUTERS

Items compared	Initial	After hour at 46.5° C.	After storage at 5° C. for				
			2 days	4 days	6 days	8 days	10 days
Motility %							
Y.C.	70	43.3	63.3	53.3	46.7	33.3	30.0
C.E. I	70	33.3	56.7	50.0	50.0	36.7	30.0
C.E. II	70	0	63.3	50.0	46.7	40.0	23.3
Rate of Motility							
Y.C.	3.8	2.5	3.3	2.5	2.3	1.8	1.0
C.E. I	3.8	1.5	2.7	2.7	1.5	1.2	0.8
C.E. II	3.8	0	2.8	2.5	1.2	1.2	0.8
Initial glucose level and loss after storage mg./100 ml.							
Y.C.	209.8	53.6	38.3	36.5	56.5	80.0	75.6
C.E. I	230.5	50.5	43.8	46.5	61.5	66.7	86.3
C.E. II	247.9	50.8	24.4	44.1	51.7	51.7	55.8
Initial lactic acid level and gain during storage, mg./100 ml.							
Y.C.	42.0	51.4	45.1	52.3	84.3	72.8	68.3
C.E. I	111.6	51.1	72.5	74.8	95.7	105.3	85.8
C.E. II	102.7	48.5	34.5	56.0	69.3	51.3	71.9

chick-embryo diluent in a more completely glycolytic metabolic system, than is the case in the yolk-citrate diluent.

As stated earlier, a diluent (C.E. II) was made from the whole embryos only and this was compared with the yolk-citrate and the whole-fertile-incubated egg diluter (C.E.I.). The eggs had been incubated for 9 days at

99.5°C. and for 1 additional day at room temperature. Only three ejaculates were involved in this study. The results are shown in table 2.

In this particular comparison, with semen samples averaging 70 percent initially motile spermatozoa, averaging 3.8 in rate of motility and with an average count of 1,035,000 spermatozoa per mm.^{3}, no significant difference in livability of the spermatozoa was observed. In fact, the spermatozoa in the yolk-citrate maintained their motility on storage as well as did those in the two other diluents. There was a difference in lactic acid gain, however, as can be seen in the table, the largest increase being in the chick-embryo I diluter.

A striking observation was that the spermatozoa in chick-embryo II diluter failed to survive the 1 hour incubation at 46.5° C., though survival was satisfactory in the other two diluters during the incubation, and was satisfactory in chick-embryo II during low-temperature storage. This is the first observation made in our laboratory where survival at incubation temperatures in a dilutor was not a satisfactory indication of the ability of the semen to survive during storage at 5° C. Another minor difference was that the methylene blue reduction times in the three diluters was: in Y.C. 8.3 minutes, in C.E. I 5.0 minutes, and in C.E. II 5.7 minutes, on the average.

The data presented in this paper show that a chick-embryo diluter made of whole-fertile-eggs incubated for 9 or 11 days is but little superior for the maintenance of motility of the spermatozoa during 5° C. storage to the usual yolk-citrate diluent.

In the artificial breeding cooperative with which the authors are most closely connected, from 200 to 500 ml. of diluent are used daily, the amount required varying with the season. The work and time required to prepare either one of the chick-embryo diluters limits their use under such circumstances, especially the chick-embryo II diluter made from the small embryos only. However, there is no evidence in this limited comparison to suggest a superiority of the diluter made from 10-day embryos only, over the yolk-citrate.

In view of these facts it is questionable whether the minor, though mathematically significant, superiority of the chick-embryo I diluent, as prepared in this study, would justify its use over the yolk-citrate, now commonly used here. Especially is this true in view of the fact that later work suggests that the yolk-citrate can be made superior to the chick-embryo I by a simple change in its composition.

Summary

Two different experiments were conducted to determine the value of a chick-embryo diluter as compared to the yolk-citrate. The chick-embryo diluent (C.E. I) made from whole-fertile-eggs incubated 9 to 11 days was

only slightly superior in maintaining livability of the spermatozoa during low-temperature storage to the yolk-citrate. The difference was observed largely with the semen samples of poorest original quality, which ordinarily would not be used in commercial artificial insemination. In a limited experiment with semen of better average quality no difference was noted in spermatozoa livability during storage between yolk-citrate, chick-embryo made from whole-fertile-incubated eggs, and a diluter made from the embryos only.

The semen samples in the chick-embryo I diluent produced slightly more lactic acid during storage than did the same semen samples in the yolk-citrate. Also, a larger proportion of the glucose lost on storage was recovered as lactic acid in the chick-embryo diluents. This fact suggests that the chick-embryo material aided in the promotion of glycolysis by the spermatozoa.

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THE LEVELS OF OVARIAN HORMONES REQUIRED TO INDUCE HEAT AND OTHER REACTIONS IN THE OVARIECTOMIZED COW

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BEFORE rational use can be made of gonadic hormones as an aid to animal husbandry practice it is necessary to determine the dosages which will produce the reactions observed in the normal animal. This is all the more important in the cow because, as a species, she deviates in several important respects from most others, and especially from the other domestic animals. She has a longer life of the corpus luteum (19 days) than is usual (10–16 days), and a shorter heat period than is usual for species of her size. She goes out of heat before she ovulates and experiences a postestrous bleeding from the uterus about 24 hours after ovulation. In order to answer the main question and to throw possible light upon the causes of these peculiarities this work was undertaken.

Experimental

Estrogen Level and Heat

A number of grade dairy heifers from 2 to 3 years old were ovariectomized through an incision in the left flank. They were then kept for 35 days or more to drain them of hormone reserves and were then injected with estradiol benzoate.

Observations were made upon their behavior and in some cases they were slaughtered at appropriate times to obtain material for histological study and for observations upon the physiology of the uterine muscle.

At first, high daily doses of estrogen were given as it was expected that the dose needed to bring the cows in heat would be proportionate to their size and in line with dosages reported for other domestic animals. However, this proved to be very wide of the mark and much lower doses had to be employed. At first the standard procedure was to inject with a given dosage daily for 6 days, but when it was realized that nearly all the cows responded to a given dose on the fourth day, or not at all, the initial injections were made near the threshold and the quantity was increased by 75 rat units per day. It was found that 250 r.u.[2] daily did not bring any cows in heat, though they showed, in some cases, proestrous phenomena such as standing near other cows or near the bull, who was not interested in them. By using the

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[2] Rat units.

gradual increase method, after a test for 6 days at 250 r.u. in some cases and averaging the dosage for the three days previous to heat, the minimal dose for producing heat was worked out for 10 heifers. This average heat inducing dose was found to be 600 ± 38 r.u. daily for 3 days. The range was from 366 to 800 r.u. and the standard deviation 178 r.u.

Two heifers failed entirely to show signs of sexual excitement. One of these failed at any level of estrogen treatment up to 5,000 r.u. daily, though she did show some mucus and a reddened, swollen vulva. A second experiment with her at 1,000 r.u. daily for 6 days, after an extended priming at lower doses, failed, although the precaution was taken to run a young bull with a painted brisket with her. This method of detecting heat was used in all the later experiments lest extremely short heats at night might have been missed. However, this was not so. It had always been difficult to decide when this heifer was in heat before she was ovariectomized. The second heifer had been easy to detect in heat prior to ovariectomy but she failed to respond to any dosage up to 70,000 r.u. daily. Her vulva never swelled or changed color, though a slight mucous discharge was recorded on three isolated days during the injections. Both these heifers were subsequently used in the progesterone experiment and were slaughtered. Their uterine muscles were entirely normal in their responses.

The sexual behavior of all the heifers before ovariectomy had been observed and rated. In general, it was noticed that, the easier it had been previously to detect heat, the lower was the level of hormone necessary to induce heat after the operation. A freemartin was brought into heat at a level of 600 r.u. daily.

Three heifers and a freemartin were used to determine the minimal level of stilbestrol which would induce heat. This was found to be 663 r.u. or 0.255 mg. daily. The range was 0.133 mg. to 0.3 mg. and the freemartin required the highest dose. A dose of 0.1 mg. daily was subminimal in all cases.

An important fact is that the duration of heat was, in nearly all cases, less than one day. At the dosages employed, up to 10,000 r.u. daily, continuous heat was never observed, and the mean length of heat, though not accurately determined, cannot be far from the average for normal cows. The cow remains in heat for the normal period and then an "estrous block" sets in. In heifers which were slaughtered after they had ceased for some days to be in heat the uterine muscle showed the typical estrous reactions and the lengths of the individual fibres were normal for estrus. It is probable, therefore, that the block occurs in the central nervous system. Zuckerman (1938) has observed a similar reaction to threshold doses in the rat.

A discharge of blood has not been obtained at any level of estrogen injec-

tion, and it does not follow estrogen withdrawal. It is well known that in primates which menstruate, bleeding can be delayed by high estrogen injections and that it follows cessation of injections (Corner, 1942). It must be remembered, however, that the type of bleeding in the two species is different. In the primates it is due to an extensive denudation of the endometrium, while in the cow it is a focal bleeding due to the breakdown of capillaries and diapedesis of the erythrocytes. The bitch has a similar type of bleeding to that of the cow, but it occurs during the proestrum and it can be induced by the injection of estrogens (Meyer and Saiki, 1931).

At levels of estrogen injection below 5,000 r.u. daily, mucin is discharged from the vagina. At levels above this no mucin is produced and the vaginal walls are dry. Preliminary histological work indicates that it is prevented by excessive keratinization of the vaginal wall at the higher levels.

An attempt was made to obtain additional information on the hormone level in the estrous cow by collecting urine from cows in heat and testing it for estrogens. Four assays were made in two heifers at two heat periods each by a method sensitive to a level of 18 r.u. per liter. In all cases the results were negative. Three heifers injected with estrogens up to 700 r.u. daily were also tested and none could be detected in the urine.

The level of progesterone needed to bring about the reactions peculiar to this hormone was between 18 and 36 rabbit units daily for 6 days, but as the method of evaluation depended upon uterine motility the evidence for this statement is presented in the next section. The low level is as surprising as is that of estrogen.

Uterine Motility

The methods used in determining the motility of the uterine muscle were the same as those used by Cupps and Asdell (1944). The amount of spontaneous activity was first found, then the following drugs were used, each on a fresh strip of muscle, pituitrin, epinephrin, arecolin, and lentin. These had been found to be the most active drugs in the normal cows.

Ovariectomy. Two control ovariectomized cows were slaughtered and the uterine motility tested. One showed slight, but irregular, contractions, but in the other there was no spontaneous activity. Response to pituitrin was positive, but small, in both cases. There was no response to epinephrin in one case and a slight contraction in the other. This is contrary to the results obtained for the uterus of the cat which, like that of the cow, contracts when the animal has been under the influence of progesterone and relaxes when it has been under the influence of estrogens. Robson and Schild (1938) found that the uterus of the ovariectomized cat relaxes when epinephrin is added to the bath. However, their two cats had been spayed for

only 15 days and the muscles were very active spontaneously. There was no response to lentin in either of the cows, while to arecoline one showed a good contraction and the other a slight one.

Estrogen treatment. Five ovariectomized cows were slaughtered after estrogen treatment, the first three after they had been brought in heat with daily doses of 20,000, 10,000 and 5,000 r.u. estradiol benzoate, and the last two with average daily doses of 366 and 450 r.u. daily. The first three were out of heat by the time they were slaughtered, while the last two were still experiencing estrus.

Spontaneous motility tended to be high in all five cows; contractions were regular in two and irregular in three. The pattern of contraction was much like that found by Cupps and Asdell (1944) for cows in heat. Three responded well to pituitrin by tonic contractions, but in two which tended to stay in tonic contractions spontaneously no response could be obtained from the contracted muscle. In all five cases epinephrin produced a marked relaxation. Lentin and arecolin produced contractions in all the strips.

Estrogen and Progesterone. Five ovariectomized cows were brought in heat with estrogen. They were then continued on a subminimal dose of 250 r.u. estradiol benzoate for six days. The reason for this was that it was wished to simulate as far as possible the conditions which probably exist during pregnancy. All were slaughtered on the seventh day. Two received, in addition to estrogen, 70 rabbit units daily of progesterone. This was the pure synthetic compound (Schering). In one there was no spontaneous activity, while the other showed high regular contractions. Pituitrin produced a marked effect as did also arecolin and lentin. Epinephrin produced good contractions.

One of the estrogen treated cows received also 35 rabbit units daily of progesterone. The spontaneous contractions of her muscle were high, but somewhat irregular. All the drugs tested produced strong contractions.

Two of the estrogen treated cows received also 18 rabbit units progesterone daily. In each case the spontaneous contractions were low and irregular. Pituitrin, arecolin and lentin contractions were well marked. Epinephrin produced a diphasic response, first a contraction, followed by relaxation. Both responses were strong.

Progesterone alone. One heifer was brought into heat with estrogen. These injections were then stopped and progesterone alone was injected for 6 days at a level of 18 rabbit units daily. She was slaughtered on the seventh day. Her spontaneous uterine muscle contractions were regular and of great amplitude. Pituitrin, arecolin and lentin responses were strong. With epinephrin she showed the diphasic response of the last group, first a contraction, followed by relaxation, and both responses were well marked.

A striking feature of all the progesterone treated cows was the strength of the response to drugs.

The diphasic response at low estrogen, low progesterone levels was believed to be due to the ability of the progesterone to produce its effect but to its inability to inhibit the estrogen. However, the diphasic response in the cow receiving low progesterone alone dose not support this view unless the estrogen used to bring the heifer into heat had a longer effect than was anticipated. More work is needed at these critical levels to decide the question. However, it is reasonable to conclude that the minimum dosage of progesterone is about 18–35 rabbit units, but it may be lower. It is interesting to note that Cupps and Asdell (1944) found a similar diphasic response in one cow at 2 days proestrum, but the amplitude was not nearly so great

In general, the results are in accord with those obtained during the cycle in the normal cow.

Length of Uterine Muscle Cells.

The mean length of the cells in the uterine muscle was obtained by the method used by Cupps and Asdell (1944), except that 25 percent nitric acid was substituted for 30 percent in the maceration as it resulted in the damage of fewer cells. The average results are given in table 1.

Table 1. LENGTH OF UTERINE MUSCLE CELLS

Group	Mean length of cells	
	Ovarian end of horn μ	Cervical end of horn μ
Ovariectomized cows (2)	100	91
High estrogen (3)	113	103
Low estrogen (2)	133	106
Low estrogen + progesterone (5)	125	108
Progesterone alone (1)	130	101

The two ovariectomized cows differed widely in their means (87 and 110μ for the ovarian end) and this rendered all differences non-significant in an analysis of variance. But if these cows were neglected the differences between treatments became significant. The levels in all groups except the ovariectomized one are close to those found by Cupps and Asdell (1944) for cows in the estrogen phase of the cycle, but the results suggest that high estrogen does not produce greatly enlarged cells, while low estrogen does. The number of animals is too scanty to decide whether progesterone helps maintain the length or whether low estrogen produces an effect in spite of

its presence. The results with normal cows would support the latter view. More data are needed on this question. In view of the fact that the cow receiving progesterone alone had as large cells as those with estrogen and progesterone it may be recalled that she also showed a diphasic response to epinephrin.

Discussion

The "estrous block" which happens when the cow has been in heat for a day or less, together with the low level of estrogen needed to produce heat gives a clue to the reason why the cow normally goes out of heat before she ovulates. Apparently the low threshold is reached fairly early in the growth of the Graafian follicle, refractiveness then sets in and the cow goes out of heat but further development of the follicle is required before ovulation.

The astonishingly low thresholds for effective action of the gonadic hormones are consistent. The cow has the lowest known level of FSH[3] in the anterior pituitary and a very low level of LH[4] (Witschi, 1940). The amount of gonadotropic hormone required for ovulation is about half that required by the ewe (Casida *et al.*, 1944), while the average dose of estrogen needed to bring the cow in heat is 1,800 r.u. compared with 3,000 r.u. in the anestrous ewe (Cole and Miller, 1935). In addition, the excretion of estrogen is extremely low both during estrus and during pregnancy as compared with other mammals.

The following relationships may be pointed out. The order of potency in descending order in FSH values of the pituitary are as follows: man, horse, hog (West and Fevold, 1940), sheep, cow (Witschi, 1940). The descending order of the length of preovulatory or heat periods is man, horse, hog, sheep, cow. Their order in excretion of estrogens per litre of urine about the time of ovulation is horse, man, hog, cow, as far as one can judge from the rather scanty evidence. Their order in requirement of estrogen to induce heat or to produce preovulatory changes in the uterus appears to be horse, man, sheep, cow. In general, therefore, there seems to be a certain parallelism throughout in the order of these species. The conclusion may be drawn that there is a general relationship between FSH content of the pituitary, estrogen requirement and excretion and length of the heat period for the species named. As data for other species become available, it will be interesting to see how they fit into the scheme, but, undoubtedly other factors, such as relative sizes and urine output, will have to be taken into account before any far reaching conclusion can be made.

The low threshold in the cow may have a bearing upon the problem of the

[3] Follicle stimulating hormone.
[4] Luteinizing hormone.

production of the freemartin. If the low threshold holds for male hormones also, it is easy to see how a small amount in the circulation may affect the development in the female twin. The suggestion is pertinent since Wislocki and Hamlett (1934) have found synchorial litter mates in the cat without modification of the gonad, while Hill (1934) found that the gonadotropic hormone content of the cat's pituitary is about 30 times that of the cow. If the relationship suggested above is correct, the threshold in the cat should be much higher than in the cow, so that it may be much harder to modify the development of the gonads.

It is also reasonable to suppose that the greater length of life of the corpus luteum of the cycle is related to the high lactogen content (Reece and Turner, 1937) of the pituitary in the cow since the corpus luteum depends for its function upon that hormone.

Recently, Moore (1944) has carefully summarized our knowledge of the freemartin condition and has pointed out two things; firstly that it seems to be peculiar to the bovine, and secondly that there is, at present, no experimental basis for the generally accepted explanation of its cause. Injection of sex-hormones into pregnant females has failed to affect the gonads of the embryos. However, the experimental approach has not been attempted upon the cow. The suggestion of threshold differences and hormone levels affords a theoretical explanation which needs to be tested.

Summary

The average level of estradiol benzoate required to bring ovariectomized heifers into heat is 600 rat units daily for 3 days. The duration of heat is usually less than one day even though the injections are continued. This low threshold is probably reached early in the development of the Graffian follicle in the normal cow. 'Estrous block,' apparently in the central nervous system, then sets in, so that the cow is out of heat before ovulation.

Mucin is produced in the vagina if the dose of estrogen is below 5,000 r.u. but not if it is above this level.

Bleeding is not produced by estrogen injection and does not follow its withdrawal.

The mean dose of stilbestrol needed to bring the ovariectomized heifer in heat is 0.255 mg.

Uterine muscle of ovariectomized heifers is relatively inert, both spontaneously and to pituitrin, epinephrin, lentin and arecolin. Uterine muscle of heifers estrogenized after ovariectomy has the same reactions as that of heifers in heat.

Progesterone in doses of 35 rabbit units or more given for 6 days produces the same reactions of the uterine muscle as those found in heifers during the

diestrum. Progesterone in doses of 18 rabbit units given for 6 days produces a diphasic response to epinephrin, first a contraction, followed by relaxation, similar to that found during proestrum.

The average length of the uterine muscle cells is greater in the estrogenized heifers than in the ovariectomized controls. Progesterone, at the levels given, does not inhibit this action of estrogen.

In domestic animals the level of FSH in the pituitary, the length of the heat period, and the estrogen threshold and excretion during heat are strikingly parallel; if one is high, the others are high, etc. The following descending order is, in general shown: man, horse, hog, sheep, cow.

The connection of the low hormone threshold in the cow with the freemartin problem is discussed.

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A METHOD FOR ESTIMATING WEANING WEIGHTS OF RANGE CALVES AT A CONSTANT AGE[1]

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FREQUENTLY it is desirable to have weights at a constant age for use in comparing animals of different parentage, production under different management practices, and similar studies. Since birth dates vary, it would be necessary to secure weights daily over a period of time to obtain actual weights at a constant age. In practice, especially under range conditions, this procedure is not feasible and usually the whole group is weighed once at weaning. The weights thus obtained vary in age at which they are taken, and in making comparisons it is frequently desirable to adjust weights for differences in age.

Convenient methods for standardizing weights of pigs (Bywaters and Willham, 1935; Whatley and Quaife, 1937) and lambs (Phillips and Brier, 1940) have been published. Data accumulated at this Station over a period of years have made possible the formulation of a method for adjusting calf weights that is simple and rapid. They also illustrate some principles useful in deriving estimating equations from original data.

Data and Discussion

Data Used

The data used in deriving the method to be described were the weaning weights of calves from the experimental range of the New Mexico Experiment Station from 1936 through 1943. The calves were dropped from March through May and were run with their dams on semiarid range where black grama was the predominant grass. Steer calves were castrated about June 1. The calves were weaned all at one time in October or early November at an average age of slightly less than seven months. A single weight was taken upon the day of weaning. Securing weights on successive days is not practical under range conditions and because of excess handling of cattle necessary to obtain them, increased accuracy would be doubtful.

Development of Method

The weights were classified by year, sex, and age of dam, and regression of weight on age calculated (table 1). The average regression of weight on age within subgroups was 1.33 pounds per day. Inspection of the data, how-

[1] Published with the approval of the Director of the New Mexico Agricultural Experiment Station. Contribution No. 36.

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TABLE 1. REGRESSION AND CORRELATION DATA ON AGE (X) AND WEIGHT (Y) OF CALVES

Source of variation	Degrees of freedom	Sums of squares and products			Regression coefficient	Correlation coefficient
		Sx^2	Sxy	Sy^2		
Subgroups	107	161,830	221,051	1,972,352	1.37	.39*
Within subgroups	755	356,956	474,196	1,719,360	1.33	.60*

* Significant at the 1-percent level.

ever, showed that the regression coefficient was positively correlated with average weight of group from which the coefficient was calculated (table 2).[3] This factor made necessary the use of a variable regression coefficient.

TABLE 2. RELATIONSHIP OF AVERAGE WEIGHT OF GROUP AT 205 DAYS AGE (X) AND REGRESSION COEFFICIENT (Y)[1]

Degrees of freedom	Sums of squares and products			Regression coefficient	Correlation coefficient
	Sx^2	Sxy	Sy^2		
29	34,485	238.24	3.24	0.0069	.72*

* Significant at the 1 percent level.

[1] Estimating equation in terms of regression coefficient b and weight W:

$$b = 0.0069W - 1.525.$$

See also footnote No. 3.

The form equation chosen for correcting weight to a standard age was

$$(1)\quad W = w + db$$

where W is the corrected weight, w the weight at weaning, d is the standard age minus the age at weighing and b the regression coefficient. The average weaning age of 205 days was used as standard age in all calculations.

The best estimate of the regression coefficient could be expressed in terms of W by the equation

$$(2)\quad b = 0.0096W - 1.525 \text{ (table 2)}$$

Substituting for b in equation 1, the estimating equation became

$$(3)\quad W = w + d(0.0069W - 1.525)$$

[3] Varying weights and coefficients were obtained by combining subgroups of each sex for 8 separate years and for 7 different aged dams. The weight of each group was adjusted to 205 days of age by its own average regression coefficient. All groups averaged around 205 days of age and little or no correction was necessary. Three important factors which influenced the rate of growth were yearly variations in feed supply, sex of calf, and age of dam. The above procedure was followed, rather than using individual subgroups, to determine whether the change in regression coefficient followed the same trend irrespective of cause of variation in weight. The regression coefficients followed the general trend in all classifications.

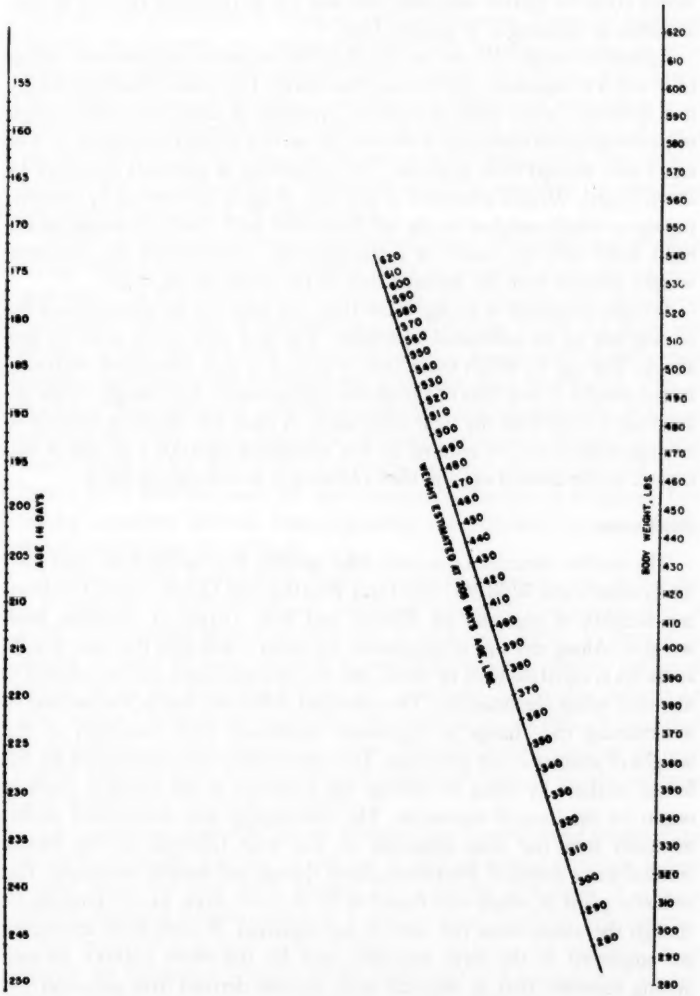

Figure 1. Nomograph for estimating weights of calves at a constant age of 205 days. Lay a straight-edge across age on the left hand scale and observed weight on the right hand scale and corrected weight is read directly from the center scale.

which could be further simplified but was left in this state because of convenience in reducing it to graphic form.

Estimated weight W can be calculated by substituting observed values of w and d in equation 3 or its simplified form. The process becomes laborious, however, when many animals are involved. A rapid convenient means of performing this operation is effected by use of a nomograph (figure 1). The chart was derived from equation 3 by adaptation of methods described by Davis (1943). Weight estimated at 205 days of age is determined by connecting age at which weighed on the left hand scale with observed weight on the right hand scale by means of a straight-edge, and reading the corrected weight directly from the inclined scale in the center of the chart.

Weight estimated at an age other than 205 days can be secured from the nomograph by an additional operation. The first step is the same as that above. The age to which correction is desired is then connected with estimated weight at 205 days of age on the inclined scale, and weight at the desired age is read from the right hand scale. A chart for adjusting directly to any age desired may be secured by first modifying equation 3 so that it will correct to the desired age and then reducing it to nomograph form.

Discussion

The method described does not differ greatly in principle from that used by Bywaters and Willham (1935) and Whatley and Quaife (1937) in adjusting weights of pigs and by Phillips and Brier (1940) in adjusting lamb weights. Along any line of regression, equation 1 will give the same results as the form equation used by them, and the two equations can be reduced to identical terms algebraically. The principal difference lies in the method of determining the change in regression coefficient with variation in the weight of animals at any given age. This relationship was determined by the former authors by using an average age intercept as the common point of origin for the lines of regression. The relationship was determined mathematically from our data (equation 2), and with reference to the former method was a means of determining both the age and weight intercepts. The common point of origin was found to be at 60.07 days, 221.01 pounds, although the values were not used in our equation. If both these intercepts are employed in the form equation used by the above authors, an estimating equation that is identical with the one derived from equation 1 is obtained.

The desirable features of the procedure used in this paper in deriving an estimating equation from original data are: (1) the form equation 1 is true whether or not the lines of regression have a common point. If there is no trend in the size of the regression coefficient with increase or decrease in

weight at a given age, the lines of regression do not tend to converge and error may be introduced by using an average intercept. When the regression lines do not converge, an average regression may be used or it may be necessary to use separate coefficients on different groups of animals, depending on the original data; (2) equation 2 provides a mathematical expression of the relationship of regression and weight at any given age if such exists. An average intercept on one axis may, or may not as in our data, give a close approximation; and (3) calculation of estimated weight is simplified by converting the estimating equation to graphic form.

When animals varying in age are weighed at one time, regression of weight on age reflects the influence of date of birth on growth rate if such influence is present. For this reason, lines of regression obtained from such data do not correspond necessarily to segments of growth curves. Under our range conditions, calves that are born late in the calving season tend to grow more rapidly, especially under adverse conditions, than those born earlier. This influence is reflected in the regression coefficients and consequently in the estimating equation. Thus, instead of estimating what an individual or group would weigh at 205 days of age, it is estimated what the weight would have been if birth date had been 205 days previous to the day of weighing. Estimating equations derived from regression along growth curves estimate weight strictly on an age basis.

In comparative studies such as effect of sire, management, etc. on weight, it is desirable to account for any difference in growth rate due to time of birth. The method described does this as explained above. In certain instances it might be more desirable theoretically to have weights estimated strictly on an age basis and account for variations due to date of birth by other means. Under range conditions this would entail the difficult procedure of establishing growth curves for calves born at different times of the year and for different years. The method of using regression of weight on age is more satisfactory for range conditions.

The regression of weight on age followed the general trend whether the variation in weight was due to sex, age of dam, or yearly variation in feed supply. Since the data were secured over a period of 8 years, it appears unlikely that any marked departure from this trend would be encountered.

The equation and chart derived from our data are not expected to be suitable for correcting weights where conditions and management are not similar to those encountered at this Station. The principles involved, however, are applicable to data from many sources and illustrate a means of adjusting weights that is simple, rapid, and more satisfactory for many purposes than methods that fail to adjust for variation in growth rate due to date of birth.

Summary

A convenient graphic method for estimating weaning weights of range calves at a constant age is presented. Weaning weights for 8 years were used to derive the estimating equation which was fitted to a nomograph chart. The equation adjusts for differences in birth dates as well as for age and is felt to be more satisfactory for some purposes than methods which do not take into account seasonal influence on growth rate.

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STUDIES ON THE CONTROL OF THE NUCHE FLY AND CATTLE TICK

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THE Nuche fly, *Dermatobia hominis* (Linnaeus, Jr., 1781), and the cattle tick, *Boophilus microplus* (Canestrina, 1887; Dönitz, 1907), are the two most destructive parasites affecting livestock in Latin America. Losses to the livestock industry in the various countries, it is estimated, run into millions of dollars annually. The Nuche fly is found principally in the intermediate altitudes of the countries bordering the Caribbean Sea, whereas the cattle tick is found in all parts of Latin America.

The object of this preliminary work was to study control measures for the Nuche fly. It was found, however, as indicated in this report, that it is possible to control both parasites with the same formula.

The Nuche fly, *Dermatobia hominis*, is known in Brazil as Berne, Ura, and Torcel, and in the other Latin American Republics by the common names of El Nuche, El Torsalo, Gusano de Monte, Gusano de Colmoyate, Gusano Macaco and Gusano de Zancudo.

The economic damage of the fly is difficult to measure. The larvae in the animal render the hides useless for leather. Many animals die from the infestation as a result of arteries cut by the larvae, or where secondary infection takes place. It has yet to be determined how seriously the larvae infestation affects the health and physical well-being of the animal. It is reasonable to suspect, however, that the loss of blood and the irritation is sufficient to lower the resistance of an animal to diseases and to lessen its ability to convert efficiently feedstuffs to milk or meat.

If one were to attach a piece of chalk to the end of the tail of an infested animal and inscribe an arc over the animal's back and sides, it would be noted that over 90 percent of the infestation is located outside the inscribed arc, or in the forequarters of the animal. Still it is not uncommon to see animals completely covered with the larvae, often huge pieces of flesh hang from the animal. The concentration of the infestation varies, often one to five larvae using the same respiratory passage.

The Life Cycle of the Nuche Fly

Most workers agree that the life cycle of the Nuche fly is as outlined below. The cycle is completed in from 120 to 135 days depending upon the temperature. It can be summarized as follows: From egg laying to the appearance of the larvae—4 to 8 days; larval period before entering the animal

[1] Turrialba, Costa Rica.

—1 to 3 days; larval period in the animal—38 to 48 days; pupa stage—70 to 72 days; life span of the adult—7 to 9 days.

The cycle is complicated by the Nuche fly having a large variety of intermediate hosts. The majority of the intermediate hosts are of the order Diptera, especially those that feed on the animal's blood or subcutaneous excretions.

The fly lays up to 400 eggs, depositing from 2 to 20 eggs on the abdomen of these intermediate hosts. It often requires several successive days to complete the laying of the eggs. From four to six days after the ovulation, the larvae begin to form in the egg and when fully developed they abandon the intermediate host when it alights upon a likely final host. Chavarría and Kumm (1939) have caught and photographed mosquitos bearing the eggs of the Nuche fly at the very moment the larvae were about to leave the egg for the final host.

The body heat of the final host helps to rupture the eggs thus releasing the larvae, which enter the animal through its hair follicles. The larva stops penetration when it gets beneath the epidermis. Here it maintains a respiratory passage to the exterior. The larvae remain in the animal about 42 days, after which they drop to the ground and continue as pupae for 72 days before becoming adult flies.

Experimental Procedure and Results

Method of Counting the Nuche Larvae

In order to establish an index of the infestation and death rate resulting from treatment, counts were made of the larvae in the animal prior to treatment. It was recognized that many larvae share common vent holes, often as many as five larvae being found using the same respiratory passage. As there was no method to determine the actual number without extracting them, each vent hole was counted and recorded as one larva.

Three men were trained to count the larvae in the animals. They spent a thirty-day practice period making larvae extractions in order to gain experience in recognizing vent holes containing live or dead, or no larvae at all.

Each animal of the control and treated groups was counted weekly by two different men. Neither man was aware of the other's count until the results were compiled. It was found that the counts of these men would not vary more than 15 percent. The average of the two counts was recorded as the total or the infestation number of the animal.

The Effect of a Rotenone Water Solution on the Control of Nuche Fly Larvae

Ten head of cattle were divided into two groups of five each. Both groups were placed in the same pasture and handled under like conditions. All

animals were weighed weekly. Group I was treated with a rotenone solution which was prepared by crushing one pound of green derris roots and extracting the rotenone content in one gallon of water for 24 hours. The solution was applied by hand on the animals with a rag and a bucket. Group II received no treatment.

The experiments began on September 29, 1944, and terminated on October 26, 1944. A storm, however, started on October 25, and so in order to observe the effects of a continuous rain on the infestation number, the trials were extended to November 2. The results of this trial are summarized in table 1.

TABLE 1. RESULTS OF USE OF ROTENONE WATER SOLUTION IN CONTROL OF NUCHE FLY LARVAE

Group	Infestation September 29, 1944	Infestation Oct. 26, 1944		Infestation Nov. 2, 1944, after one week of storm	
		Infestation	Percentage of No. on Sept. 29	Infestation	Percentage of No. on Sept. 29
	number	*number*	*percent*	*number*	*percent*
I	300	210	70	96	32
II	378	511	135	285	75

The physical appearance of the two groups was notably different at the end of the trial. In the group that received treatment the larvae scabs and wounds were drier and cleaner smelling and the animals were not sensitive to probing of the infested areas. In the control group there was evidence of pus and blood, a general smell, and the animals were extremely sensitive to the probing of the infested areas.

The rotenone solution had a definite effect on the infestation rate of group I. Also, it killed approximately 30 percent of the larvae in the animals. There was a pronounced effect in the healing of the wounds and some evidence of the reduction of tick infestation. In the control group the larvae count was 35 percent more than the original infestation number.

The storm which began on October 25 and ended on November 2 was a continuous rain that totaled 15½ inches. As can be seen in table 1, this storm lowered the infestation number in both groups.

The Effect of DDT and DDT-Rotenone Solutions on the Nuche Fly

Fifteen head of cattle were weighed and divided equally into three groups of five animals each. A seventy-acre pasture was divided into two parts by a double fence so that the control group could not contact the treated groups.

These groups were switched weekly so that average experimental conditions would be the same for the control and treated groups. Nuche larvae counts were made weekly at the time of treatment by the method previously described. The groups were treated weekly as follows: Group I was sprayed with 4 percent DDT solution in kerosene. The sprayer was a "Flit gun" of a type used in controlling house flies. Each animal received an average of 25 cc. of this solution. This was enough to cover the animal twice. Group II was sprayed first with approximately one quart of a rotenone solution made by crushing one pound of green derris roots and extracting the rotenone content for 24 hours in one gallon of water. Following the application of the rotenone each animal was sprayed with 20 cc. of the 4 percent DDT solution in kerosene. Group III received no treatment. The results are summarized in table 2.

TABLE 2. RESULTS OF STUDY OF EFFECT OF D.D.T. AND ROTENONE SOLUTIONS ON THE NUCHE FLY

Group	Infestation October 18, 1944	Infestation on Dec. 20, 1944	
		Infestation	Percentage of the Oct. 18 Infestation
	number	*number*	*percent*
I	347	180	51.8
II	313	51	16.2
III	134	208	155.2

The 4 percent solution of DDT in kerosene (group I) showed evidence of effecting control of the Nuche fly. The wounds of the animals, however, did not show signs of healing more rapidly than normally. This group was infested with cattle ticks and at the end of three treatments of seven day intervals, all ticks were killed.

The combination of DDT and rotenone sprays lowered the infestation number of group II and was apparently better than either the rotenone or the DDT solutions separately. This group was also infested with ticks which died after the second treatment.

Spray Tests with a DDT-Rotenone Spray Solution

From the results obtained with the DDT-rotenone combination, the following single spray solution was devised and tested: Formula No. I.A.S. ah 9-107; 1 gm. DDT powder, 14 c.c. kerosene, 40 gms. chopped green derris roots, 6 gms. neutral soap, and 400 c.c. soft water. It has given ex-

cellent results in Nuche fly[2] and tick control measures (Mohler, 1930). A 400 c.c. application of this solution as fine spray is sufficient to cover and protect an animal.

A number of beef animals each covered with approximately 2 ticks per square inch of body surface and full of tick wounds and scabs were sprayed with a fine sprayer. A single application of approximately 400 c.c. of solution I.A.S. ah 9-107 was administered to each animal.

All animals were examined at 24-hour intervals. It was found that this solution was completely effective against the tick. The maximum killing time of the solution varied between 48 and 96 hours.

The results of these spray tests give a method for controlling the cattle tick more applicable to many areas in Latin America than the cattle bath. A single spray application capable of killing all visible ticks will make it possible to use a mobile control system in controlling ticks on cattle in transit. It will assist the small livestock man in areas that cannot afford a cattle bath. Deaths resulting from bathing cattle under tropical conditions will be averted.

Summary and Conclusions

Data from preliminary experiments using rotenone and DDT in the control of the Nuche fly and the cattle tick may be summarized as follows:

A rotenone water extract was found to be useful in controlling the infestation rate of the Nuche fly in cattle although not completely effective in killing the Nuche larvae in the animal. This solution had little apparent effect on the cattle tick. However, it exhibited healing properties.

A 4 percent DDT powder dissolved in kerosene showed an apparent effect in Nuche fly control. It also controlled the cattle tick after three applications, one week apart. No apparent healing properties were shown by this solution. No data were obtained on probable fly mortality due to DDT

Best results in Nuche fly control were obtained with a double spray of 4 percent DDT powder in kerosene and a rotenone water solution made by using green crushed derris roots. All cattle ticks were destroyed after two applications of these solutions, one week apart. Similar healing properties, shown by the rotenone solution, were apparent.

Observations made at the Institute indicate some seasonal differences as to the rate of infestation of the Nuche fly.

A negative relationship between the number of the larvae of the Nuche fly in the animal and a period of constant rainfall was found.

A single spray solution of DDT and rotenone was developed and tested. It was found to be effective against the cattle tick with a single

[2] Unpublished data of the Institute.

spray application. This spray also shows promise in Nuche fly control measures. No visible effects of the toxicity of this solution to animals have been encountered to date. Extreme caution must be exercised until the full toxic effects of DDT have been worked out.

Spraying and bathing animals alone cannot be depended upon to effect a complete control of the Nuche fly and cattle tick. Good animal management must be practiced along with the control measures taken. Because fly populations are generally greatest in shady, swampy areas, much control can be exercised by cleaning pastures and reducing shade to a minimum. Water holes must be kept open and free of weeds.

The use of open sided sheds is recommended to serve as shade and weather protection for live stock. These can be kept clean and sprayed for fly control.

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THE MONTHLY PROTEIN AND PHOSPHORUS CONTENTS OF TWO IMPORTANT RANGE GRASSES OF SOUTHERN NEW MEXICO[1]

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MUCH of the semiarid mesa land lying in the south-central part of New Mexico along the Rio Grande has an average annual precipitation of from 8½ to 10 inches. Over half of this precipitation usually occurs in July, August, and September, the primary growing season during which most of the range grass is produced. At the end of the growing season these mature grasses are usually subjected to very light precipitation throughout the winter and spring months, as shown by the 25-year average given in table 1.

TABLE 1. PRECIPITATION RECORDS, BY MONTHS

Month	Year									25-year average
	1936*	1937	1938	1939	1940	1941	1942	1943	1944	
	inches	*inches*	*inches*	*inches*	*inches*	*inches*	*inches*	*inches*	*inches*	*inches*
January	—	.00	.35	1.60	.02	2.45	.26	.46	.35	.35
February	—	.65	.39	.01	.93	.73	.36	.00	.63	.38
March	—	.44	.06	.51	.06	1.25	.00	.10	.01	.39
April	—	.00	.00	.12	.21	1.17	.56	.00	.00	.20
May	—	.87	.00	.00	.16	.70	.00	.32	.24	.57
June	—	.67	1.15	.29	1.58	.69	.29	1.01	.06	.56
July	—	.36	4.29	1.96	2.72	.58	1.05	1.77	1.32	1.75
August	.81	1.04	.03	1.17	.50	2.20	3.40	.63	2.44	1.81
September	.74	3.17	3.05	.92	1.12	5.92	1.42	1.38	1.43	1.34
October	.57	.89	.09	.52	1.04	1.39	.54	.00	1.22	.89
November	1.20	.00	.02	.44	1.08	.33	.00	.91	1.25	.46
December	.04	.48	1.01	.39	.26	.85	.81	1.08	.68	.63
Total, annual		8.57	10.44	7.93	9.68	18.26	8.69	7.66	9.63	9.33

* Rain gauge was not installed until July 1936.

With light precipitation during the winter and spring, the wintering and leaching processes are held to a minimum, with the result that many of the grasses still retain much of their feeding value throughout that part of the year. This usual light winter precipitation is one of the most important factors which contribute to the rather high wintertime feeding value of these

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range grasses. However, the occurrence of comparatively heavy late fall and winter rains may entirely change the situation and leave a rather depleted forage.

The reduction in the nutrients of the grasses left standing on the ranges during the winter cannot be attributed entirely to the processes which are called wintering and leaching. There is a cycle through which plants normally pass when the growing season ends and the dormant season begins. The mature plant has stored a considerable percent of its nutrients in the seed and these in turn are shattered and lost to the plant. The action of the sun causes some of the food constituents, especially certain vitamins, to be destroyed; while the wind may cause leaves to be torn from the stalk and lost. When these more or less normal losses are not accompanied by excessive fall and winter precipitation the forage is a valuable carbohydrate roughage for wintering livestock.

Material

Two of the most important range grasses of the southern New Mexico area are black grama (*Bouteloua eriopoda*) and mesa dropseed grass (*Sporobolus flexuosus*). These two species were selected for a study of their nutritive properties. Black grama, one of the very palatable grasses for livestock, grows on the mesas, hills, and dry open ground of large areas of range land in Texas. New Mexico, Arizona, and southern Utah. The upright stems of this grass remain green for a distance of 4 to 6 inches from their bases throughout the winter. This small amount of plant activity of the black grama grass undoubtedly results in increased protein values and also in moderate carotene values, during the winter and spring months. Breeding cows grazing entirely on black grama during the winter are provided with sufficient carotene for maintenance and in most years a large part of the increased amounts of carotene needed for gestation and lactation may be provided.

Mesa dropseed grass is found on the mesas of western Texas, New Mexico, Arizona, southern Utah, and southern California. This grass grows in bunches, usually to a height of 20 to 30 inches, and is very palatable throughout the growing season. The above-ground growth of mesa dropseed grass dies with the advent of freezing weather and loses most of its carotene within a very short time.

Data and Discussion

Protein

The monthly protein contents of black grama and mesa dropseed grasses are given in table 2. The primary growing season, as has been intimated,

occurs during July, August, September, and October. Grasses often contain as high as 16 percent of protein on an air-dry basis during this growing season. The protein values for these two grasses during the winter and spring months are lower than those obtained during the late summer and fall.

TABLE 2. CRUDE PROTEIN CONTENT OF BLACK GRAMA (*BOUTELOUA ERIOPODA*) AND MESA DROPSEED (*SPOROBOLUS FLEXUOSUS*), BY MONTHS*

Month	Year									Average
	1936**	1937	1938	1939	1940	1941	1942	1943	1944	
	Black Grama									
	percent	percent	percent	percent	percent	percent	percent	percent	percent	percent
January	—	5.97	5.03	3.58	5.05	5.45	5.98	3.54	4.40	4.88
February	—	5.79	4.98	3.27	5.01	5.19	5.67	3.54	4.53	4.75
March	—	6.12	4.72	3.55	4.70	4.61	5.13	4.05	4.49	4.67
April	—	6.16	5.21	4.04	5.10	5.01	—	3.97	4.57	4.87
May	—	6.08	4.70	3.73	4.70	6.59	5.53	3.45	5.05	4.98
June	—	5.99	4.79	3.56	4.04	5.27	4.57	3.96	5.87	4.76
July	—	6.82	12.49	3.45	6.52	5.98	6.21	3.80	5.52	6.35
August	5.92	6.34	5.05	6.85	9.58	7.38	6.04	6.81	8.06	6.89
September	12.07	10.88	7.75	7.25	7.20	7.21	6.64	6.77	7.50	8.14
October	9.13	8.00	4.70	6.70	6.90	7.34	5.26	6.64	—	6.83
November	5.99	5.42	3.79	5.71	6.06	6.11	4.14	5.99	—	5.40
December	5.62	5.11	3.70	5.36	6.02	6.31	4.66	5.05	—	5.23
	Mesa Dropseed									
January	—	5.43	4.81	3.14	4.53	4.48	5.31	3.84	—	4.51
February	—	6.01	4.41	2.79	3.91	4.88	4.22	2.85	—	4.15
March	—	4.55	3.57	2.13	4.39	4.66	4.07	3.41	—	3.83
April	—	4.98	3.53	2.13	3.91	4.57	—	2.42	7.81	4.19
May	—	5.86	4.83	3.27	7.78	6.20	4.74	2.72	6.93	5.29
June	—	8.04	3.83	3.47	4.35	7.29	3.58	2.67	6.51	4.97
July	—	6.86	8.49	2.77	10.32	6.20	5.91	—	7.12	6.87
August	12.20	7.82	6.88	5.80	9.53	7.82	6.81	10.31	13.54	8.97
September	15.93	12.02	9.23	8.04	7.12	8.09	8.63	8.06	10.61	9.75
October	9.74	9.75	6.22	8.04	5.89	6.81	5.30	9.57	—	7.67
November	8.03	7.61	3.96	4.48	6.02	5.53	5.48	5.22	—	5.79
December	5.48	5.16	3.61	5.18	4.83	5.10	3.97	4.53	—	4.73

* All analyses are on an air-dry basis.

** Collection of samples was begun in August 1936.

From an inspection and comparison of data in table 2, it will be noted that black grama does not contain as much protein during growth as the mesa dropseed, but that it contains much more than the dropseed during the dormant season. There are factors which influence growth and curing and may

greatly affect the protein content of the grasses. The amount of rainfall, its distribution over the growing season, the occurrence or absence of excessive winter precipitation and variations in temperature seem to be some of the main factors influencing the protein content of the grasses during the various months of the year.

There were two instances of very low protein in the grasses, and these occurred in the winters and springs of 1938–39 and 1942–43. The 1938–39 period was most severe and can be termed as one in which severe leaching with respect to protein occurred. The growing season of 1938 was unusual in that two separate growths of grass were produced. The heavy precipitation of June and July produced one crop of grass. This was followed by a very dry August. Heavy September rains produced another crop which matured before frost. This heavy growth of grass was subjected to comparatively heavy December and January rains which seemed to wash and leach the protein out leaving the grasses with a very low content of protein. In southern New Mexico, black grama and mesa dropseed normally loses approximately 30 to 50 percent, respectively, of their protein on the average, between the end of the growing season and March. Through this severe leaching, this species of grama and the mesa dropseed lost 50 and 74 percent respectively, of their protein, during the same period, In contrast to these low protein values, the black grama had an average protein content of 4.96 percent for March when the values for the two low winters are not included. This March protein content seems fairly high for this time of the year and may illustrate the idea of the southwestern grasses curing well on the stalk.

A study of pure stands and species of grass is important, and it is necessary that consideration be given to the species of grass and browse that are being grazed at a particular time of the year, in order to determine the adequacy of the ration. Mesa dropseed grass, as has been mentioned, is grazed during the summer and fall and is generally used much less extensively during the winter and spring months. When favorable moisture conditions occur, palatable weeds and other annuals appear on this range, sometimes as early as February. This early desert herbage is often many times higher in protein and phosphorus than the dry range grasses.

The heavy winter rainfall of 1938–39 which leached out an unusually large part of the protein of the grasses, also provided the moisture to grow many palatable weeds which appeared in March and in part relieved the protein and phosphorus situation. These early spring weeds contained from 9 to over 20 percent of protein on an air-dry basis. The blossoms of *Yucca elata* contained from 13 to 18 percent of protein on the same moisture basis. Chamiza (*Atriplex canenscens*) and sand sagebrush (*Artemisia filifolia*) con-

tained approximately 10 and 11 percent, respectively, of crude protein at this time of the year.

Phosphorus

The pattern of the phosphorus content of the range grasses is very similar to that of protein with the higher content occurring during the growing season and with much lower values during the dormant season. This change from the condition found in the fall seems to be attributable largely to changes occurring with the maturity of the grasses and their weathering in the dormant season. The average phosphorus losses between those of the growing season and the following March was 43.2 percent for black grama and 67.8 percent for mesa dropseed grass. During the first four months of 1938, 1939, and 1943 the grasses were lower than usual in phosphorus content.

If a 1,000-pound breeding cow is considered to consume 20 pounds of dry matter from the forage, a percentage of .095 on an air-dry basis would provide 8.5 grams of phosphorus daily. This much total phosphorus is needed during the winter when the cows are dry. During the period, March to October, the amount of phosphorus should be increased to 12.5 grams or .113 percent on an air-dry basis, to satisfy the increased needs for this element during lactation. Reference to the phosphorus content of these two grasses given in table 3 reveals that the phosphorus needs are not met with the exception of during a short period at the peak of the growing season.

There is a factor in the nutrition of cattle during the winter and spring on ranges in this region which may compensate for the low phosphorus content of the most important grasses. This, as has been mentioned before, is the abundance or scarcity of annual weeds during this period, depending upon moisture conditions. The value of these weeds when they do become sufficiently large for grazing, should not be underestimated. This type of forage was not included in the project in which much of these data were collected but was covered by a later study which has been completed and the results from which have been published (Knox, Benner, and Watkins, 1941).

It was found that during the late winter and spring period cattle were consuming weeds which were high in phosphorus, containing in some cases as much as ten times the quantity of this element found in an equal weight of the available range grasses. It was also noted that these annual plants were sometimes consumed in comparatively large quantities. The phosphorus contents of some of these annual weeds and browse are given on an air-dry basis. The early spring weeds contain from .216 to .434 percent of phosphorus while *Yucca elata* blooms contain .140 to .493 percent of this element. Big sagebrush (*Artemisia tridentata*) and sawtooth rabbit brush

(*Chrysothamnus vicidiflorus serrulatus*) contain an average of .315 and.246 percent phosphorus respectively. Silver sagebrush (*Artemisia cana*) and sand sagebrush contain an average of .209 and .221 percent phosphorus respectively. Winterfat (*Eurotia lanata*) and chamiza contain approximately the same amount of phosphorus, .144 percent.

TABLE 3. PHOSPHORUS CONTENT OF BLACK GRAMA (*BOUTELOUA ERIOPODA*) AND MESA DROPSEED (*SPOROBOLUS FLEXUOSUS*), BY MONTHS*

Month	Year									Average
	1936**	1937	1938	1939	1940	1941	1942	1943	1944	
	Black Grama									
	percent	*percent*	*percent*	*percent*	*percent*	*percent*	*percent*	*percent*	*percent*	*percent*
January	—	.075	.036	.061	.071	.061	.069	.043	.045	.058
February	—	.088	.034	.042	.046	.060	.069	.042	.059	.055
March	—	.070	.035	.042	.055	.052	.083	.038	.058	.054
April	—	.065	.041	.067	.052	.062	—	.043	.049	.054
May	—	.072	.078	.068	.064	.083	.075	.051	.057	.069
June	—	.062	.044	.109	.058	.083	.060	.041	.141	.075
July	—	.061	.135	.046	.060	.095	.086	—	.052	.076
August	.063	.066	.078	.086	.110	.117	.068	.089	.100	.086
September	.149	.124	.113	.106	.084	.119	.115	.077	.082	.108
October	.148	.110	.090	.104	.096	.135	.105	.088	—	.110
November	.083	.077	.074	.108	.079	.124	.055	.091	—	.086
December	.064	.044	.088	.091	.092	.093	.065	.050	—	.073
	Mesa Dropseed									
January	—	.069	.022	.024	.042	.072	.063	.049	—	.049
February	—	.094	.043	.049	.030	.063	.062	.039	—	.054
March	—	.032	.025	.038	.032	.058	.039	.047	—	.039
April	—	.038	.023	.036	.031	.067	—	.045	.086	.047
May	—	.053	.057	.043	.053	.080	.054	.023	.071	.054
June	—	.062	.024	—	.040	.129	.043	.033	.081	.059
July	—	.075	.088	.039	.107	.149	.079	—	.056	.085
August	.105	.084	.122	.065	.128	.145	.081	.115	.193	.115
September	.151	.155	.188	.126	.111	.178	.162	.131	.136	.149
October	.138	.144	.165	.125	.111	.132	.114	.143	—	.134
November	.124	.129	.105	.091	.110	.091	.052	.057	—	.095
December	.066	.066	.081	.096	.088	.101	.063	.046	—	.076

* All analyses are on an air-dry basis.

** Collection of samples was begun in August 1936.

Although certain phosphorus requirements have been set up for this area by Watkins (1937), Knox, Benner, and Watkins (1941) and Watkins (1943), it is felt that additional research should be conducted on the requirements

of this element, especially the minimum requirements. A study of the results of this Station indicates that the estimated phosphorus intakes from all sources, including the usual grasses and occasional weeds, often fall short of these requirements. The feeding of mineral supplements mixed with salt which provide 2½ to 3 grams of phosphorus daily usually brings the phosphorus intake up near these requirements. However, the usual phosphorus deficiency symptoms such as depraved appetite, brittle bones, and creeps have not been noticed on the ranch of the New Mexico College of Agriculture and Mechanic Arts. A thorough study has been made of the phosphorus intakes and it seems that the livestock in this area must utilize that element very efficiently. It may be that in this arid climate with a high percent of days with bright sunshine, the ultraviolet rays of the sun greatly assist in the utilization of phosphorus.

The data presented by Black and co-workers (1943) from the King ranch project in Texas show the average phosphorus content of 203 samples of forage to have been .076 percent. The average phosphorus content of 260 samples of the most important species of grass reported from the New Mexico Station is .088 percent. The phosphorus levels reported from these two stations are very similar, the Texas values being slightly lower; especially the winter values. Outbreaks of creeps have been reported from this Texas area, while at the New Mexico Station smaller gains are usually the only ill effects. It is believed that these phosphorus levels are very close to the levels where clinical symptoms develop for cattle in this southwestern region and that further study of the data of these two stations, along with consideration of associated factors, might give information on the minimum amounts of phosphorus which would prevent the occurrence of creeps and other deficiency symptoms.

Blood Phosphorus Determinations and Production Records

The inorganic phosphorus of the blood plasma has been determined on from 24 to 32 head of Hereford breeding cows every 28 days since 1937. These were kept continuously on the experimental range where the average annual precipitation is only about 9 inches. The average inorganic phosphorus for cows receiving additional calcium and phosphorus supplement over a three-year period during the critical months of December, January, Febuary, and March was 3.24 mg. per 100 ml. of blood plasma on a calculated total phosphorus intake of 8.45 grams. Cows in a similar pasture during the same months with no mineral supplements had inorganic phosphorus values of 2.16 mg. on a calculated phosphorus intake of 6.9 grams. The standards for beef cattle call for a blood plasma phosphorus of at least 4 mg. of phosphorus per 100 ml. of blood plasma and for a total phosphorus intake of

approximately 12 to 15 grams (Mitchell and McClure, 1937). The severe leaching which occurred during the winter 1938–39 lowered the phosphorus intake of the mineral-fed cattle to approximately 7.4 grams, with an average inorganic phosphorus value of 3.00 mg. When the feed phosphorus drops during the period of December to March inclusive, the inorganic phosphorus will often drop as low as 2.00 mg. even with the feeding of 2½ to 3 grams of phosphorus supplement. However, plasma phosphorus values as low as this seldom extend over more than a month in the mineral-fed cattle. The nonmineral group had a phosphorus intake of approximately 5.9 grams and an inorganic phosphorus value of 2.02 mg. These are the lowest values that have been secured on this range. The appearance of early weeds and annuals in March of 1939 relieved the phosphorus situation. These 12 nonmineral-fed cows later that year had 10 calves and lost 2 at birth. The other 8 were weaned at 7 months of age, with an average live weight of 467 pounds. This weaning weight without the use of mineral supplements may seem good for a southwestern range, but the addition of phosphorus supplements on this range usually increases the calf crop to above 90 percent with an average increase of 30 to 45 pounds in the live weight of the calf at weaning time.

Breeding cows on this range have given good production results when during the critical winter period the calculated phosphorus intake has been no lower than 8.5 grams and the inorganic phosphorus of the blood plasma has not fallen below 3 mg. Even though the phosphorus intakes are lower than recommended, the phosphorus requirements are apparently being met for range cattle in this area as judged by performance. The cattle weights for different ages have compared very favorably with the growth curves of steers fed for maximum growth without fattening as published by Moulton *et al.*, (1923). The gain in weight during the first year on this range is much more rapid than that given by these workers but according to unpublished data it takes approximately 7 years for the breeding cows to reach maximum weight. The final weight for these breeding cows seems to fluctuate between 1,000 and 1,100 pounds while in production.

Summary

The monthly protein and phosphorus contents of mesa dropseed and black grama grass are given covering a period of 98 months.

Moderately high protein values, especially for black grama, are usually found during the winter, owing largely to the light precipitation during this season. Abnormal winter rains may leach the grasses to such an extent that they become quite low in protein and phosphorus by early spring.

The phosphorus deficiency is more severe and of longer duration than that

of protein. Reference to the phosphorus content of the two grasses studied reveals that the phosphorus needs of range beef cattle are not met, with the exception of during a short period at the peak of the growing season.

Breeding cows on this range have given good production results when the calculated phosphorus intake has been no lower than 8.5 grams and the inorganic phosphorus of the blood plasma has not fallen below 3 mg. during the critical winter period.

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YEARLY DIFFERENCES IN GROWTH OF NAVAJO AND CROSSBRED EWE LAMBS

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Introduction

NAVAJO Indians derive the major portion of their livelihood from sheep. A relatively large amount of wool is used in the hand manufacture of rugs, while a great many animals are used for food. The surplus wool and lambs are sold on the market, and constitute important sources of cash income.

The semi-arid condition of the Southwest makes rigorous demands upon the sheep raised there. Consequently, a breeding program designed to increase the productivity of Navajo sheep must give constant attention to the reaction of the sheep to their environment. The purpose of this paper is to present results of a study in which the growth of Navajo and crossbred ewe lambs in different years were compared.

Materials and Methods

In a previous paper (Blunn, 1944) data were presented on the weights of all Navajo lambs born during the middle two weeks of May each year from 1938 to 1941 at the Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, N. Mex. From these original data the weights of the Navajo ewe lambs have been extracted and compared with the weights of crossbred ewe lambs that had been born during the same period.

The ewe lambs of this study formed the major part of the foundation stock of four closed lines that were organized in the fall of 1941. The Navajo ewe lambs were used in two of these lines. The crossbred ewes were divided between the other two lines and were produced from the following matings: 50 percent from Corriedale ♂ × Navajo ♀, 25 percent from Romney ♂ × Navajo ♀, 4 percent from Columbia ♂ × Navajo ♀, 1 percent from Rambouillet ♂ × Navajo ♀, and 20 percent were backcross lambs produced by mating ewes of the preceding four crosses with Navajo rams. In the breeding program the identity of the various crosses is lost, for they are being merged into a common type in the F_2 and subsequent generations. Furthermore, statistical analyses showed no significant difference in growth between the various kinds of crossbred animals produced in any one year, and not all of the crosses were made each year. Therefore, all crossbreds have been considered as a group each year.

[1] This study was conducted at the Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, N. Mex., under authority of the Bankhead-Jones Act, in cooperation with the Office of Indian Affairs, United States Department of the Interior.

The reader is referred to the former paper (Blunn, 1944) for a description of the management of the flock, method of weighing, description and condition of the range, and amount of precipitation during the period covered by this study.

Data and Discussion

Single and twin lambs have been combined because the effect of birth type was the same for both the Navajos and crossbreds. The numbers of Navajo and crossbred lambs and the mean weights of the several ages are presented in table 1. There was less yearly variation in birth weight for both groups of lambs than there was at any of the later ages. It is also apparent that the range in mean weights of the Navajo lambs was less than the range for the crossbred animals at each age.

TABLE 1. NUMBER AND MEAN WEIGHTS OF NAVAJO AND CROSSBRED EWE LAMBS FOR EACH AGE OF LAMBS WEIGHED, 1938–1941

Year and breed of lambs	Lambs	Age					
		Birth	4 weeks	8 weeks	12 weeks	16 weeks*	20 weeks
	number	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
Navajo							
1938	112	7.8	22.8	33.8	42.9	50.8	56.8
1939	133	7.9	21.4	31.2	39.8	47.9	55.8
1940	83	7.3	19.4	29.5	42.2	—	53.3
1941	41	7.8	23.0	36.4	47.8	56.2	63.4
Total or average	369	7.7	21.6	32.2	42.2	50.3	56.4
Crossbred							
1938	35	7.8	21.2	31.6	39.3	46.7	51.9
1939	43	8.8	23.0	32.5	40.7	49.1	55.8
1940	88	8.1	20.7	30.2	41.4	—	52.1
1941	141	8.2	25.9	40.0	51.5	60.4	67.5
Total or average	307	8.2	23.5	35.2	45.7	56.0	59.6

* No weight secured for this age in 1940.

A direct comparison of the weights of the Navajos and crossbreds shows that at birth the crossbreds were heavier in three of the four years, while the two groups weighed the same in the fourth year. In 1938 the Navajo lambs were heavier than the crossbred lambs from 4 to 20 weeks, while in 1939 the crossbred lambs were heavier until they were 16 weeks of age. At 20 weeks both groups weighed the same, but the crossbreds were more variable. In 1940 the crossbred lambs were heavier from birth to 8 weeks, but the Navajos were heavier from 12 to 20 weeks. Only in 1941 were the crossbred lambs consistently heavier at all ages than the Navajo lambs.

The analyses of variance given in table 2 follow the method given by

Snedecor (1940). They show that the crossbreds varied more both between years and within years than the Navajos, except at birth. From 4 to 20 weeks of age the mean squares for the crossbred lambs were consistently greater than the corresponding mean squares of the Navajos. This was true not only for the variance between years, but also the variance within years. At birth, however, the variance between years was slightly greater for the Navajos than it was for the crossbred lambs.

TABLE 2. ANALYSIS OF VARIANCE OF WEIGHTS BY AGES OF NAVAJO AND CROSSBRED EWE LAMBS, 1938–1941

Source of variation	Degrees of freedom	Mean squares					
		Birth	4 weeks	8 weeks	12 weeks	16 weeks[1]	20 weeks
Navajo							
Between means of years	3	7.5**	219.1**	582.2**	704.5**	1099.5**	958.2**
Within years	365	1.4	15.2	31.8	48.3	56.1	62.5
Crossbred							
Between means of years	3	6.8*	573.9**	2054.1**	2926.3**	3852.7**	5501.7**
Within years	303	1.8	22.8	38.3	65.9	75.9	84.2

[1] Degrees of freedom are 2 and 285 for the Navajos, and 2 and 216 for the crossbreds at this age.
* $P=0.05$, significant.
** $P=0.01$, highly significant.

Using Fisher's (1934) technique of intraclass correlation as extended by Winsor and Clarke (1940), the theoretical portion of variance attributed to differences between years was determined. These results are given in table 3, where they are expressed as the percentage of total variance. Also included in the table are the data for all Navajo lambs (rams and ewes) that had been analyzed and published previously (Blunn, 1944).

TABLE 3. PERCENT OF VARIANCE ATTRIBUTED TO DIFFERENCES BETWEEN YEARS

Source of variation	Age					
	Birth	4 weeks	8 weeks	12 weeks	16 weeks	20 weeks
	percent	*percent*	*percent*	*percent*	*percent*	*percent*
Navajo ewe lambs	4.1	12.7	15.8	12.8	16.3	13.4
Crossbred ewe lambs	3.3	23.9	40.7	36.1	40.5	45.6
All Navajo lambs[1]	1.2	8.8	13.1	13.1	21.6	14.2

[1] See Blunn (1944) for original data.

In table 2 of the paper dealing with all the Navajo lambs (Blunn, 1944), the two lines, between and within years, were inadvertently transposed, hence erroneous information was given. It will be noted that the two groups of data (table 2, page 44 of Blunn, 1944, and the last line of table 3 in the present paper) differ slightly. This difference was brought about by the two methods of analysis. In the former case the portion of variance attributed to within years sources of variation was expressed as a percentage of the actual total variance. The between years portion of variance was obtained by subtraction. In the present instance the mean square for the between years source of variation was reduced to its component parts and expressed as a percentage of the theoretical total variance in a population where every individual is from a different year.

From table 3 it is seen that the agreement between all Navajo lambs and the Navajo ewe lambs is quite good, except at 16 weeks of age. The difference at that age was probably brought about by the weaning of the ram lambs as they reached sexual maturity. In both sets of data an average of 14.2 percent of variance from 4 to 20 weeks of age was attributed to differences between years.

Yearly differences had a more pronounced effect on the crossbred lambs than they had on the Navajos. At 4 weeks of age the percent of variance in weight attributed to differences between years was approximately twice as large for the crossbred ewe lambs as it was for the Navajo ewe lambs. At 20 weeks of age the portion of variance due to differences between years was 3.4 times as great for the crossbreds as it was for the Navajo ewe lambs.

An average of 37.4 percent of the variance from 4 to 20 weeks of age was attributed to differences between years for the crossbred lambs in contrast to the 14.2 percent for the Navajo lambs.

It was to be expected that the crossbred population would be more variable than the Navajos within any one year. The crossbreds, however, would not necessarily vary more between years unless their response to the environment was different from that of the Navajos. The data were analyzed so that the breed × year interaction could be determined (Yates, 1934). The results of this analysis showed that the interaction of breed × year was highly significant at all ages. This means that the Navajo and crossbred lambs did not react in the same manner to the environment.

The data presented in table 3 are in agreement with this statement. They are also borne out by the growth rates of the two groups of lambs. During the drouth years of 1938 through 1941, the rate of growth of the Navajos was greater than that of the crossbreds. In 1941, however, a year of above-normal precipitation, the crossbreds grew somewhat more rapidly than the Navajos, especially between birth and four weeks of age.

As Cooper (1939) and Blunn (1943) have pointed out, the Navajos have had about 400 years in which to become adapted to the region. On the other hand, the improved rams used in the crossbreeding program came from the Northwest and Northern intermountain states. They were not adapted to either the Southwestern ranges or climate, and have undoubtedly introduced many factors which were not present in the Navajos. These factors, especially those affecting growth, have apparently operated in such a manner as to disrupt the equilibrium which the Navajo sheep have reached with the environment of the region. Also, the nature of growth has been changed in the crossbred lambs. The Navajos are a relatively small, rapidly-maturing animal. By the time the ewes enter the breeding flock at 18 months of age, they have attained 96 percent of their mature weight. The crossbred ewes are slightly larger than the Navajos, and are also somewhat slower maturing; they do not reach their mature weight until they are about 2.5 years of age.

Summary

Groups of Navajo and crossbred ewe lambs raised at the Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, N. Mex., have been weighed at intervals of four weeks from birth to 20 weeks of age each year from 1938 to 1941, inclusive.

A comparison of the weights of the two groups of lambs showed that the crossbreds varied more than the Navajos from year to year at all ages. From the analysis of variance the percent of total variation in weight due to differences between years was determined. From 4 to 20 weeks of age this averaged 14.2 percent for the Navajos, and 37.4 percent for the crossbreds. The reasons for this difference in reaction to the environment are discussed.

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THE ACCURACY OF MEASUREMENTS AND WEIGHTS OF SHEEP

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OBJECTIVE measures of size and body form are desired in many experiments with sheep, including studies of growth, inheritance and nutrition. Weight is almost universally used and some attempts have been made to use measurements to determine size of skeleton, amount of muscling, and degree of fatness. Obviously, it is often difficult to interpret measurements in terms of desirable body form or carcass characteristics, and this is a problem upon which much study is yet needed. However, there are definite advantages, in many experiments, in having at least a few objective measures that can be used in interpreting results. The measures that should be taken will depend upon the objects of the experiment, and also upon the accuracy with which the measurements can be obtained. Measurements that cannot be duplicated with reasonable accuracy obviously would be of little value.

Several studies of the accuracy of measurements and weights of sheep have been made, during recent years, in connection with various experiments conducted by the Bureau of Animal Industry. The results are summarized in this paper.

Material and Methods

Direct measurements with metal calipers and a steel tape measure, as well as weights, were obtained on five groups of animals, and measurements from photographs were obtained on a sixth group. Each group contained 11 animals and each measurement was taken 11 times on each animal. Measurements were taken to the nearest ½ centimeter, and weights to the nearest ½ pound. The groups were as follows:

Group I. Eleven mature Rambouillet ewes, measured when the fleeces were about 1 to 1½ inches long, at the U. S. Sheep Experiment Station, Dubois, Idaho. Measurements were taken over the fleece, with the wool compressed closely under the calipers.

Group II. Four Corriedale, 4 Rambouillet and 3 Lincoln ewes, measured just after shearing, at the U. S. Sheep Experiment Station, Dubois, Idaho.

Group III. Eleven mature Hampshire ewes, measured just after shearing, at the Beltsville Research Center, Beltsville, Maryland.

Group IV. Eleven Rambouillet lambs, measured when the fleeces were about 1 to 1½ inches long, at the U. S. Sheep Experiment Station, Dubois, Idaho. Measurements were taken over the fleece with the wool compressed closely under the calipers.

Group V. Eleven Hampshire yearling ewes, measured just after shearing, at the Beltsville Research Center, Beltsville, Maryland.

[1] The authors are indebted to G. W. Brier, formerly junior statistician, for assistance in the statistical calculations and to C. E. Terrill, animal husbandman, for assistance in taking the measurements on groups I and IV.

Group VI. Eleven mature Navajo ewes, photographed at the Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, New Mexico just after shearing. Each ewe was placed in a close-fitting wire cage, and photographed 11 times from the side and 11 times from directly overhead. Photographs were projected to life size, as determined by a ruler in the picture, and measurements were obtained from the projected image.

The measurements taken are shown in figure 1. The measurements were obtained at various times and with different problems in mind, hence com-

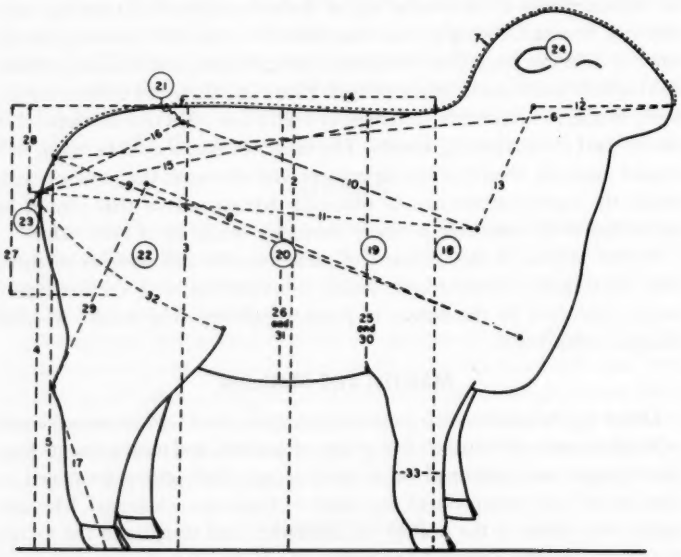

Figure 1. Diagram showing where measurements were taken. 1. Height at withers. 2. Height at back. 3. Height over hook bones. 4. Height at pin bones. 5. Height at base of dock. 6. Length, nose to dock. 7. Length, nose to dock, over head. 8. Length, shoulder point to hip. 9. Length, shoulder point to dock. 10. Length, mid-front of scapula to hook bones. 11. Length, mid-front of scapula to pin bones. 12. Length of head, nose to angle of jaw. 13. Length of neck, angle of jaw to mid-front of scapula. 14. Length, front of withers to hook bones. 15. Length, front of withers to pin bones. 16. Length, hook bones to pin bones. 17. Length, rear shank. 18. Width, at shoulders (numbers of points at which width measures were taken are in circles). 19. Width at chest, just behind shoulders. 20. Width of middle, widest point. 21. Width of hook bones. 22. Width of legs. 23. Width of pin bones. 24. Width of head, in front of ears. 25. Depth of chest. 26. Depth of middle, deepest point. 27. Depth of twist, from back over hook bones. 28. Depth from back over hook bones to pin bones. 29. Depth from hip to hock.30.Circumference of chest (heart girth). 31. Circumference of middle, largest point. 32. Circumference of legs, from stifle to stifle, over vulva. 33. Circumference of foreshank, at middle.

parable measurements were not obtained in all studies. Obviously, some measures, such as circumferences, could not be obtained from photographs (group VI). The data were analyzed by the method of calculation described by Fisher (1925) under the general title Analysis of Variance, and used by Lush and Copeland (1930) in a study of the accuracy of measurements of dairy cattle.

Results

Results of studies on the six groups of animals described above are summarized in table 1. The standard deviations and coefficients of variation given apply to the variation due to inconsistencies of measurement. These variations include those caused by changes in position of the sheep, and errors of the operator resulting from failure to always locate points of measurement exactly or by varying the pressure applied on the calipers. The taking of measurements and weights on groups I and IV required several days and naturally this tended to increase the variation classified as due to inconsistencies of measurement, particularly for weight, which would naturally be expected to vary more than measurements from day to day. A study of the data brings out several points of interest.

Measurements obtained from photographs are generally unsatisfactory In most cases the coefficient of variation is larger than for comparable measurements on sheared animals. The measures are not true indications of the size of the animal, owing to the facts that it is almost impossible to keep an animal that is not accustomed to confinement in position so that its body is at right angles to the camera, definite points are difficult to locate on a projected image, and images enlarged to life size tend to break down optically. Phillips and Dawson (1936) found direct measurements more accurate than photo-measurements of swine.

Measurements of animals in fleece are generally less accurate than those on animals just after shearing. This result was anticipated, of course. In this respect, the data in table 1 merely serve to show the relative amount of inaccuracy caused by attempting to measure an animal when in fleece. Some additional variation was undoubtedly introduced in the results with groups I and IV, owing to the fact that measurements and weights were obtained over a period of 12 days as compared with only 1 or 2 days in other groups. This probably introduced more error in weights than in measurements. Certain measures, such as height at withers, height over hook bones and width of hook bones, were taken with reasonable accuracy on animals that were in fleece. The same applies to circumference of chest and of middle, but true measures of circumference are difficult to obtain, owing to packing of wool under the tape measure. Also, a measurement such as circumference of middle might vary more from day to day than circumference of chest,

TABLE 1.—SUMMARY OF RESULTS OF STUDIES TO DETERMINE ACCURACY OF MEASUREMENTS OF SHEEP

Measurement[1]		I. Range ewes, in fleece			II. Range ewes, sheared			III. Farm ewes, sheared			IV. Range lambs, in fleece			V. Farm yearlings, sheared			VI. Navajo ewes, sheared			
No.	Name	Mean	S.D.[2]	C.V.[3]	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Mean	S.D.	C.V.	Actual measure	Mean from photos	S.D.	C.V.
1.	Height at: Withers	69.7	1.18	1.7	67.3	.86	1.3	61.5	.87	1.4	59.6	1.09	1.8	60.9	.82	1.4	65.6	60.2	1.06	1.8
2.	Back	—	—	—	67.5	.96	1.4	61.6	.92	1.5	—	—	—	60.3	.80	1.3	66.3	61.4	1.03	1.7
3.	Hook bones	69.1	1.28	1.8	68.4	.73	1.1	62.3	1.02	1.6	59.1	1.40	2.4	61.7	.88	1.4	67.3	63.1	1.02	1.6
4.	Pin bones	—	—	—	54.2	.95	1.7	50.2	1.04	2.1	—	—	—	50.3	1.01	2.0	54.0	49.9	1.24	2.5
5.	Base of dock	56.6	2.51	4.4	—	—	—	—	—	—	49.7	2.27	4.6	—	—	—	—	—	—	—
6.	Length: Nose–dock	111.7	4.06	3.6	—	—	—	—	—	—	93.8	3.84	4.1	—	—	—	—	—	—	—
7.	Nose–dock	133.0	2.85	2.1	—	—	—	125.4	1.51	1.2	110.9	2.83	2.6	119.8	1.98	1.6	—	—	—	—
8.	Shoulder–hip	66.6	2.14	3.2	—	—	—	66.0	1.60	2.4	56.5	1.67	3.0	62.7	1.48	2.4	—	—	—	—
9.	Shoulder–dock	70.7	1.94	2.7	—	—	—	—	—	—	59.4	1.71	2.9	—	—	—	—	—	—	—
10.	Scapula–hook bones	—	—	—	52.6	1.54	2.9	52.8	1.05	2.0	—	—	—	50.6	1.07	2.1	49.5	46.7	1.55	2.9
11.	Scapula–pin bones	—	—	—	73.0	1.46	2.0	73.3	.94	1.3	—	—	—	70.4	1.15	1.6	66.5	60.4	1.69	2.8
12.	Head	—	—	—	21.1	.36	1.7	21.0	.47	2.2	—	—	—	19.2	.59	3.1	20.0	17.4	1.32	7.6
13.	Neck	—	—	—	20.1	1.19	5.9	19.9	.92	4.6	—	—	—	18.0	1.08	6.0	20.1	18.5	1.54	8.3
14.	Withers–hook bones	47.9	1.68	3.5	—	—	—	—	—	—	39.2	1.65	4.2	—	—	—	—	—	—	—
15.	Withers–pin bones	66.0	1.64	2.5	—	—	—	—	—	—	54.6	1.69	3.1	—	—	—	—	—	—	—
16.	Hook bones–pin bones	18.1	1.29	7.2	—	—	—	—	—	—	15.4	1.11	7.2	—	—	—	—	—	—	—
17.	Rear shank	30.6	.81	2.7	28.6	.45	1.6	25.9	.68	2.6	27.9	.73	2.6	25.5	.71	2.8	27.9	25.0	.83	3.3
18.	Width at: Shoulders	24.4	1.10	4.5	23.3	.50	2.2	26.4	.44	1.7	20.1	1.09	5.4	26.0	.41	1.6	19.4	18.4	.76	4.1
19.	Chest	24.8	1.12	4.5	23.6	.73	3.1	26.5	.67	2.5	19.9	1.15	5.8	25.6	.60	2.4	19.2	18.4	.80	4.4
20.	Middle	31.6	1.52	4.8	28.1	1.01	3.6	34.8	.73	2.1	25.0	1.41	5.6	31.9	.54	1.7	27.5	23.2	.85	3.6
21.	Hook bones	19.8	.33	1.7	20.5	.49	2.4	22.1	.67	3.0	14.6	.31	2.1	20.1	.70	3.5	18.2	17.5	.81	4.6
22.	Legs	28.0	1.44	5.2	—	—	—	—	—	—	23.5	1.40	6.0	—	—	—	—	—	—	—
23.	Pin bones	16.2	.79	4.9	6.4	.32	5.1	6.2	.34	5.4	12.4	.51	4.1	5.4	.47	8.7	—	—	—	—
24.	Head	—	—	—	10.6	.45	4.2	10.9	.30	2.7	—	—	—	10.3	.31	3.0	—	—	—	—
25.	Depth of: Chest	33.6	.81	2.4	31.8	.51	1.6	30.8	.33	1.1	27.4	.90	3.3	29.4	.36	1.2	30.8	29.6	.48	1.6
26.	Middle	—	—	—	34.3	.65	1.9	36.0	.49	1.4	—	—	—	32.4	.49	1.5	33.8	32.2	.61	1.9
27.	Twist	10.0	1.41	14.1	22.6	.96	4.2	20.1	.98	4.9	8.4	1.03	12.3	21.8	.87	4.0	—	—	—	—
28.	Back to pin bones	15.0	1.19	7.9	—	—	—	—	—	—	11.4	1.34	11.7	—	—	—	—	—	—	—
29.	Hip to hock	32.4	1.20	3.7	—	—	—	—	—	—	27.7	1.15	4.2	—	—	—	—	—	—	—
30.	Circumference: Chest	106.8	2.30	2.2	92.6	1.02	1.1	96.6	1.09	1.1	87.9	2.33	2.6	92.9	.89	1.0	—	—	—	—
31.	Middle	119.8	2.17	1.8	103.2	1.93	1.9	117.6	1.59	1.4	100.3	2.35	2.3	107.4	.97	.9	—	—	—	—
32.	Legs	66.5	2.23	3.4	58.7	1.63	2.8	57.2	1.40	2.4	57.2	2.00	3.5	58.2	1.18	2.0	—	—	—	—
33.	Foreshank	9.6	.29	3.0	8.4	.36	4.3	9.0	.10	1.2	8.4	.36	4.3	8.9	.09	1.1	—	—	—	—
34.	Body weight	118.1	2.70	2.3	122.4	.38	.3	146.8	.78	.5	66.8	1.52	2.3	121.3	.24	.2	—	—	—	—

[1] Measurements are in centimeters, weight is in pounds.

[2] Standard deviation, of variance owing to inconsistencies of measurement, in centimeters.

[3] Coefficient of variation. Small apparent inconsistencies result from rounding of figures.

because of differences in fill or gas formation in the rumen. Measurements of animals in fleece may sometimes be desirable, such as on weaning lambs that are to be marketed, but whenever feasible, measurements should be taken on sheared animals, if maximum accuracy is to be attained.

Range ewes are more difficult to measure than farm ewes, owing to the fact that they are less accustomed to being handled and it is difficult to hold them in a standard, natural position. However, when reasonable care is exercised, they can be measured with about the same degree of accuracy as farm ewes. This is indicated by a comparison of the coefficients of variation of groups II and III. Of 22 comparable measurements, 12 were more accurate on farm sheep, 9 more accurate on range sheep, and in one case there was no difference. The coefficient of variation for circumference of foreshank is quite large in group II, and is probably a result of the failure of range shearers to trim the legs as carefully as was done with the sheep in group III.

The measurements that are most accurate and appear to measure characters than experimenters may wish to evaluate are as follows: (1) Height at withers; (11) Length from mid-front of scapula to pin bones; (18) Width at shoulders; (25) Depth of chest; (26) Depth of middle; (30) Circumference of chest; (31) Circumference of middle; (33) Circumference of foreshank. If a comparison of length of body and length of rump, or width of shoulder and width of chest, is desired, the following measures could be added: (10) Length from mid-front of scapula to hook bones; (19) Width at chest. The above measurements should be sufficient to indicate size of skeleton and thickness of body, insofar as these can be determined from measurements, and should suffice in most experiments until more is known about the relation between measurements and carcass and other qualities. Other measurements might be added, as the needs of an experiment require. In general, the measures taken should be limited to those for which specific use is planned.

Data were obtained on one additional measure, not recorded in table 1, on groups I and IV. This was circumference of one leg, taken at approximately the mid-point between hip joint and point of hock. The means were 28.8 and 26.2, and the coefficients of variation were 6.7 and 7.3, respectively. The considerable variation in this measurement was apparently due to the difficulty in locating an exact point to measure.

Weight was reasonably accurate in all groups and the coefficient of variation was 0.5 or below in the three sheared groups. The error was greater in the unsheared groups, the coefficient of variation being 2.3 for both range ewes and lambs. Most, if not all, of the additional variation in weights of the unsheared animals was probably due to day-to-day variation in actual weights, since data on these groups were obtained over a longer period than was required for the sheared groups.

[1] Measurements are in centimeters, weight is in pounds.
[2] Standard deviation, of variance owing to inconsistencies of measurement, in centimeters.

Summary

Results of six studies to determine the accuracy of measurements of sheep are presented. Accuracy of weight was also determined in five of these studies. Measurements that appear to be most accurate, and at the same time measure characteristics which experimenters may desire to study, include height at withers, length from mid-front of scaupla to pin bones, width at shoulders, depths of chest and middle, circumferences of chest and middle, and circumference of foreshank.

Measurements obtained from animals in fleece or from enlarged photographs are generally less accurate than direct measurements on sheared animals.

The difficulties of interpreting measurements in terms of carcass or other values are pointed out. Results of this study indicate that many measurements can be taken with reasonable accuracy, and some external measurements will undoubtedly be of use in interpreting the results of many experiments. It should be recognized, however, that relatively little has been done to determine the relation between external measures and scores and the characteristics of the carcass or other measures of the real productive ability of the animal. Obviously, there is need for much study on this phase of measuring performance. To avoid unnecessary work, the measurements taken in any study should be limited to those for which definite use is planned in the specific experiment under consideration, until more is known of the relation between external measures and merit of the animal.

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RESPONSE OF ANESTROUS DOES AND EWES TO PREGNANT MARE'S SERUM DURING TWO CONSECUTIVE SEASONS

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THE optimal period for natural mating of most breeds of goats and sheep is approximately three months, with less activity displayed over an additional three or four months. Numerous attempts have been made to induce these animals to breed during the period of natural sexual quiescence. Pregnant mare's serum (PMS) has been used extensively in such tests, but with highly conflicting results. Cole and Miller (1933) reported the successful use of PMS in the induction of estrus, but Cole (1937) stated that subsequent tests with the same preparation yielded highly variable results. The literature reporting results of many workers, recently reviewed by Phillips, Fraps and Frank (1945), bears out the experience of Cole. Similar findings, obtained during the years 1942 and 1943, are summarized in the present report. In addition to emphasizing the variability of results obtained with PMS, attention is called to several factors which have perhaps not received the attention due them in accounting for variability of reaction to PMS by does and ewes.

Materials and Methods

The initial results, obtained in 1942, and with which the later work is compared in this paper, were reported by Phillips, Simmons and Schott (1943), and Frank and Appleby (1943).

The PMS[3] used for the work in 1943 was rechecked for potency and found to contain more than 50 r.u. (rat units) per cc. Certain luteinizing preparations, and in a few instances progesterone, were given 24 hours or later following PMS administration. Since such injections had no apparent effect these secondary treatments may be disregarded in the present work. The PMS was injected subcutaneously.

The goats used in the work in 1943 included 33 does and 5 bucks. Part of the does were used in more than one group listed in table 1. Some of these animals had been used during 1942 for hormone injections, while the others were either young does just reaching breeding age or were normal breeding animals taken from the milking herd. The goats were of the Toggenburg and

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[3] The PMS used was "Gonadin," supplied by Cutter Laboratories, Berkeley, California.

Saanen breeds. Dates of hormone injections ranged from June 19 to August 18 in 1942, and from May 19 to September 17 in 1943.

The sheep consisted of 31 ewes and 10 rams. Some of the ewes are included in more than one group in table 2. They were of the Down breeds (mostly Hampshires, with a few Shropshires and Southdowns). These animals had been used for hormone injections during their 1942 anestrous season, and all were of active breeding age. During their natural breeding season, following earlier injections, all ewes had exhibited normal estrus cycles and most of them became pregnant when bred. Some of the ewes with late pregnancies were aborted in order that they might be used in this work. Those pregnant 100 days or more were regularly aborted by giving diethylstilbestrol. They were given 2 mg. the first day, 1 mg. the second day; abortion

TABLE 1. COMPARISON OF THE INCIDENCE OF ESTRUS INDUCED IN ANESTROUS DOES BY PMS DURING TWO CONSECUTIVE SEASONS (1942, 1943)

Group	Initial injection (r.u.)	Second[a] injection (r.u.)	Third[a] injection (r.u.)	Fourth[a] injection (r.u.)	Number of does	Does in estrus	
						Number	Percent
Tests of 1942							
A-1	150×3[b]				1	1	100
A-2	150×4[b]				1	1	100
A-3	150×6[b]				1	1	100
B-1	200	400			9	9	100
B-2	200	200	400		3	3	100
C-1	400				2	2	100
C-2	400	600			1	1	100
C-3	400	600	600		2	1	50
					20	19	95
Tests of 1943							
D	50×3[c]				3	2	67
E-1	150×3[b]				9	0	—
E-2	150×3[b]	400			3	0	—
E-3	150×3[b]	400	50×3[c]		1	1	100
F-1	200	400			15	0	—
F-2	200	400	400	50×3[c]	6	1	17
G-1	400				11	1	9
G-2	400	50×3[c]			8	1	13
					56	6	11

[a] The injections were made at 18 or 20 day intervals.
[b] Multiple injections were given on alternate days.
[c] Multiple injections were given on consecutive days.

followed on the third day. Those less than 100 days pregnant did not abort with this treatment. Dates of injections ranged from July 11 to August 18 in 1942, and from June 4 to September 3 in 1943.

The does and ewes were tested for the presence of estrus by turning normal males with them. Animals that came in estrus were bred. An exploratory laparotomy was performed on numerous individuals 5 or 9 days after PMS injections to determine the response of their ovaries to the hormone.

TABLE 2. COMPARISON OF THE INCIDENCE OF OVULATION INDUCED IN ANESTROUS EWES BY PMS DURING TWO CONSECUTIVE SEASONS (1942, 1943)

Group	First injection			Second injection[a]			Third injection[a]			Total		
	r.u.	Number ewes		r.u.	Number ewes		r.u.	Number ewes		Lapar.	Ovulating	
		Lapar.[b]	Ovul.[c]		Lapar.	Ovul.		Lapar.	Ovul.		No.	Percent
Tests of 1942												
A-1	250	2	2							2	2	100
2	500	1	1							1	1	100
3	250×2[d]	1	1							1	1	100
4	250+1000	1	1							1	1	100
5	500×2	1	1							1	1	100
6	125+50×6	1	1							1	1	100
7	125+50×3	1	1							1	1	100
B-1	250			50	3	3				3[e]	3	100
2	250			250	3	3				3	3	100
										14	14	100
Tests of 1943												
C-1	25	6	0	25×5	6	0				12	0	0
2	50	5	0	50	4	0				9	0	0
D-1	250	12	8	250	12	5				24	13	54
2	250	6	2	250	5	0	250×2	6	4	17	6	35
3	300	7	6	300	8	4				15	10	67
										77	29	38

[a] The injections were made at 16 day intervals.
[b] Lapar. = Laparotomized.
[c] Ovul. = Ovulating.
[d] Multiple injections were given on consecutive days.
[e] One ewe not examined conceived.

Results

Goats

The experiments with goats for 1943 were outlined to supplement the work of 1942, referred to above. As a preliminary check on the condition of their ovaries, before any injections were made, three does were selected at

random for exploratory laparotomies. Their ovaries appeared to be in a resting state.

Results of tests with does during 1942 and 1943 are summarized in table 1. The animals injected during 1942 fall into three groups (A, B and C) depending upon initial level of injection, and a number of sub-groups representing animals subjected to differing subsequent treatments. Thus group A consisted of 3 does injected with 150 r.u. on alternate days until estrus supervened; this required 3 injections for 1 doe, 4 for a second, and 6 for the third. Group B consisted of 12 does, none of which came in estrus following the initial injection of 200 r.u. PMS; following a 20 day interval 9 does (group B-1) were injected with 400 r.u. and the remaining 3 with 200 r.u. PMS (group B-2). All the does receiving 400 r.u. (group B-1) came in estrus, thus terminating the tests. Since none of the does receiving 200 r.u. came in estrus following the second injection, they were given a third injection of 400 r.u.; all came in estrus. Group C, consisting of 5 animals, was broken down into its sub-groups by similar procedures, except that one animal in group C-3 did not come in estrus following her third injection, at which time the tests were terminated.

The injection of does during 1943 differed from the procedures of 1942 mainly in that injections were terminated before all animals came in estrus (group E-3) being a minor exception). However, a number of direct comparisons can be made between the results of the two years. Thus of the 13 does composing group E, only one came in estrus following initial injection (150×3), compared with one out of three animals similarly injected during 1942 (group A). Comparison of the 9 does of group B-1 with the 21 animals of group F through the interval of second injections shows that all the does came in estrus during the 1942 tests, none during the 1943 tests. Groups C and G may be similarly compared following initial injections; 2 out of 5 does came in estrus in 1942, one out of 19 during 1943.

One point of interest in connection with the tests of 1943 is that 5 of the 6 animals exhibiting estrus did so following the injection of 50 r.u. to each ewe daily for three consecutive days, in 2 does as initial injections, in the other 3 as secondary injections. However, 18 does were injected in this manner, of which the 5 just noted represent only 28 percent.

Sheep

The experiments with ewes for 1943 were outlined to determine the minimum and optimum dosage of PMS that would induce ovulation comparable with 1942, and to determine the possibilities of inducing estrus with ovulation.

The results of 1942 and 1943 tests with ewes are summarized in table 2. The ewes injected in 1942 (with the exclusion of animals killed 48 hours after injection) fall in two groups; those that were given single or multiple daily injections (group A), and those that received injections at 16 day intervals (group B). Each group was divided into sub-groups according to subsequent treatment. When examined after PMS injections 13 of the ewes had ovulated and another became pregnant when bred at this time.

Two ewes out of 14 came in estrus following initial injections and 4 ewes out of 6 following a second injection after a 16 day interval.

The tests with ewes during 1943 differed from those of 1942 principally in that in some instances both slightly smaller initial doses and sub-minimal doses of PMS were given. These ewes fell into two groups: those receiving sub-minimal doses required to induce ovulation (group C), and those receiving doses considered adequate to induce ovulation (group D). However, a few direct comparisons may be made. After comparable initial injections all three ewes in groups A-1 and A-7 (in 1942) had ovulated in comparison with 16 out of 25 in groups D-1, 2 and 3 (in 1943). A similar comparison may be made between groups B and D following a second injection made at a 16 day interval, where all 6 animals had ovulated in 1942 and only 9 out of 25 had ovulated in 1943.

Following initial injections two ewes out of 14 came in estrus in 1942, while none out of 26 came in estrus in 1943. On a similar basis four out of six ewes came in estrus following a second injection made at a 16 day interval in 1942, as compared with two out of 26 in 1943.

Another point of interest in the 1943 tests was the occurrence of estrus in 3, and ovulation in 4, out of 6 ewes following a third 16 day interval injection of 250 r.u. X2. This was the largest dose of PMS given at one interval and the only group of ewes injected at three periods (group D-2).

Discussion

The main point of interest in the tests reported here lies in the contrasting results of 1942 and 1943, a contrast involving both ovulation in the ewes and estrus in the does. For reasons already noted, it does not seem possible to attribute the differing responses to differences in the PMS preparations used nor to the manner of its administration. The composition of the flocks used also rules out both genetic and age factors as the basis for the different responses in the two years' tests. The possibility of antihormone effects likewise appears remote.

If we may admit that the factors just noted cannot account for the differing results of 1942 and 1943, we must seek an explanation in physiological,

nutritional or environmental variables. The existence of physiological differentials is self-evident in the observed responses, but the existence of such differences during the interim of PMS injections most probably represents the resultants of antecedent influences of nutritional or environmental origins.

Zavadovskii (1939) obtained conflicting results during two consecutive seasons with the use of PMS to induce estrus and ovulation in ewes. During the first year a high percentage of the animals came in estrus and conceived, while practically none came in estrus or conceived the following year. He attributed the erratic results to a difference in nutrition as influenced by prevailing weather conditions.

During the present work the spring of 1942 was marked by good rainfall, which assured an abundance of grass during most of the season; spring rainfall was light during 1943 and the growth of grass was somewhat below normal. While such differences cannot be accurately assessed, there seems little doubt that the 1942 season was more favorable in point of green feed, and possibly with respect also to dried feeds. Conditions of light, temperature and humidity also differed during the two years. Without attempting at this time to evaluate these variables in any quantitative sense, their roles in the enhancement or retardation of PMS action in the induction of ovulation or estrus seems highly probable.

In this connection a recent report by Hertz and Sebrell (1944) is of interest. It was found that an adequate intake of folic acid is required for normal response of the chick oviduct to stilbestrol. Chicks on a diet deficient in pantothenic acid, however, exhibited a good response to injected stilbestrol. There is at least a suggestion here that specific vitamins or vitamin balances may be involved in the response of an organ or system to the sudden and drastic demands imposed upon specific functions following administration of PMS. This does not imply that the animals were on a deficient diet by usual standards. Nutritional levels were almost certainly higher during the anestrous (summer) months than during the periods of normal estrus; the question is simply whether the induction of ovulation or estrus during the usual anestrous period is in part conditioned by nutritional factors which may be of little or no importance during the seasons of normally exhibited estrus and ovulation.

Other extraneous factors, such as temperature or change in the length of light day, may also be involved in the variable response to injection of PMS. Bissonnette (1941) has shown that breeding cycles in goats may be induced by artificially reducing length of day, or inhibited by lengthened days; under the conditions of his experiments he concluded further that the annual

temperature cycle "is not a major factor in environmental control of sexual or breeding cycles in goats." It is at least conceivable that differences in the light cycle from season to season may predispose ewes and does to greater or lesser reactivity to PMS. Under normal conditions it would be difficult to rule out similar effects which might be attributed to temperature differences from year to year, although Bissonnette's results indicate little influence from this source.

In the last analysis the physiological condition of the animal determines its response to PMS and similar gonadotropes. The conflicting results obtained following the administration of PMS to does and ewes suggest the desirability or studying the response of these animals under differing, accurately controlled environmental and dietary conditions. It should not prove difficult, once the conditions necessary for consistently favorable responses are known, to accommodate these to the practical demands of breeding during the season of sexual quiescence.

Summary

Erratic results were obtained in two similar experiments conducted in 1942 and 1943 in which the action of pregnant mare serum (PMS) was studied in connection with the induction of estrus in does and estrus accompanied by ovulation in ewes during their anestrous seasons.

During the 1942 season 19 out of 20 does (95 percent) came in estrus following one or more injections of PMS, whereas during the 1943 season 6 out of 56 (11 percent) exhibited estrus following comparable treatment.

In 1942, 6 out of 14 ewes that were given PMS came in estrus and all had ovulated when examined by laparotomy. Following comparable dosages of PMS in 1943, only 2 out of 26 came in estrus and 9 out of 25 had ovulated when examined.

Attention is called to the possibility that environmental or nutritional variables are involved in the divergent responses often exhibited by ewes and does to the administration of PMS.

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NEWS AND NOTES

The Executive Committee of the American Society of Animal Production has decided to cancel the annual meeting for 1945. It seemed highly improbable that permission could be obtained from O.D.T. to hold the meeting, and in view of the existing travel and hotel situations, it was decided that no request should be made. Since no meeting will be held, no abstracts of papers will be called for. The space that would normally be used for abstracts in the November issue will be used for complete papers, thus making it possible to clean up some of the existing backlog of manuscripts.

W. G. Kammlade, of the animal husbandry department, University of Illinois, Urbana, Illinois, has been selected by the Executive Committee as secretary-treasurer of the American Society of Animal Production, for the remainder of the current term, replacing W. V. Lambert. Dr. Lambert resigned, owing to pressure of work in his new position as assistant research administrator in the U. S. Department of Agriculture.

E. A. Trowbridge, head of the animal husbandry department at the University of Missouri will become dean of the college of agriculture and director of the experiment station on September 1.

V. A. Rice, head of the animal husbandry department and of the division of agriculture at Massachusetts State College is now dean of the school of agriculture at that institution. The trustees recently designated the divisions as schools, and the heads of divisions as deans.

James Watson, former instructor in animal husbandry at the South Dakota State College, was killed in action in the South Pacific where he was serving with the United States Army Air Corps. He was graduated from Montana State College in 1937, and joined the South Dakota State College staff shortly thereafter.

Arthur H. Walker (lieutenant colonel) who was on leave from the animal husbandry department of the University of Arizona, was killed in action in Italy last February.

Hugh C. McPhee, of the Bureau of Animal Industry in Washington, D. C., was a member of the technical staff attending the Inter-American Conference on Agriculture at Caracas, Venezuela in July.

Claude Harper has been made head of the animal husbandry department at Purdue University, succeeding Frank G. King, who will continue as a member of the staff.

S. S. Wheeler, a member of the animal husbandry department, University of Wyoming, was made head of the same department at the Colorado State College of Agriculture, effective July 1, succeeding R. C. Tom, who resigned to engage in commercial work.

Gordon C. Cairns, head of the animal husbandry department, University of Maine, has gone to the University of Maryland, as head of the dairy department.

I. W. Rupel, former associate professor of dairy husbandry, University of Wisconsin, has been made head of the dairy department at Texas A. & M. College.

W. L. Stangel, professor of animal husbandry at Texas Technological College, Lubbock, Texas, will become dean of agriculture of that institution, effective September 1.

A. E. Darlow, head of the animal husbandry department, Oklahoma A. & M. College, W. E. Carroll, head of the animal husbandry department, University of Illinois, Earl Weaver, head of the dairy husbandry department, Michigan State College, and J. R. Dawson of the U. S. Bureau of Dairy Industry, have been granted leave of absence to take charge of courses in their respective fields in the educational centers being set up by the War Department in England and France for the instruction of American soldiers, particularly those awaiting discharge and transportation.

A. L. Baker, formerly of the U. S. Range Livestock Experiment Station staff, Miles City, Montana, became superintendent of the Iberia Livestock Experiment Station, Jeanerette, La., June 16, succeeding A. O. Rhoad, who resigned to become assistant director of the experiment station sponsored by the Institute of Inter-American Affairs in Turrialba, Costa Rica.

Phil W. Ljungdahl, formerly agricultural agent in Chase County, Kansas, has been appointed to succeed J. J. Moxley as extension animal husbandman in that State.

F. A. Ralston, formerly agricultural agent in Beaverhead County, Montana, has been made extension animal husbandman in that State to succeed E. P. Orcutt, resigned.

E. T. Robbins, who has been extension livestock specialist at the University of Illinois since 1923 and has a total of 30 years service to his credit, plans to retire September 1.

E. J. Thompson, for many years head of the animal husbandry department, North Dakota Agricultural College, and more recently manager of Tyrconnel Farms, Knoxville, Maryland, died suddenly March 28.

F. W. Burns, extension dairy specialist, Alabama Polytechnic Institute, and former livestock specialist at that institution, died suddenly on April 11.

M. L. Buchanan, formerly a member of the respective animal husbandry staffs at West Virginia University, the University of Georgia and the University of Idaho, and more recently in commercial work, has now joined the animal husbandry staff at North Dakota Agricultural College.

P. L. Kelly, associate professor of animal husbandry and associate in dairying at the University of Arkansas, has resigned.

B. W. Fairbanks, chief in swine husbandry in the animal husbandry department, University of Illinois, resigned effective May 1 to become head of research work for the American Dry Milk Institute, Chicago.

Malcolm H. Kerr, of the animal husbandry department, University of Connecticut, has resigned to accept a position on the animal husbandry staff at the University of Tennessee.

A. S. Severson, at one time a member of the animal husbandry staff, North Dakota Agricultural College, has accepted a position with the American Aberdeen-Angus Association as fieldman for the Northwestern States.

D. G. Hotchkiss is now assistant animal husbandman, North Dakota Agricultural College.

Berry Duff, former county agent at Sonora, Texas, and graduate of the University of Wyoming, has been appointed to a position in the wool division office of marketing services, War Food Administration.

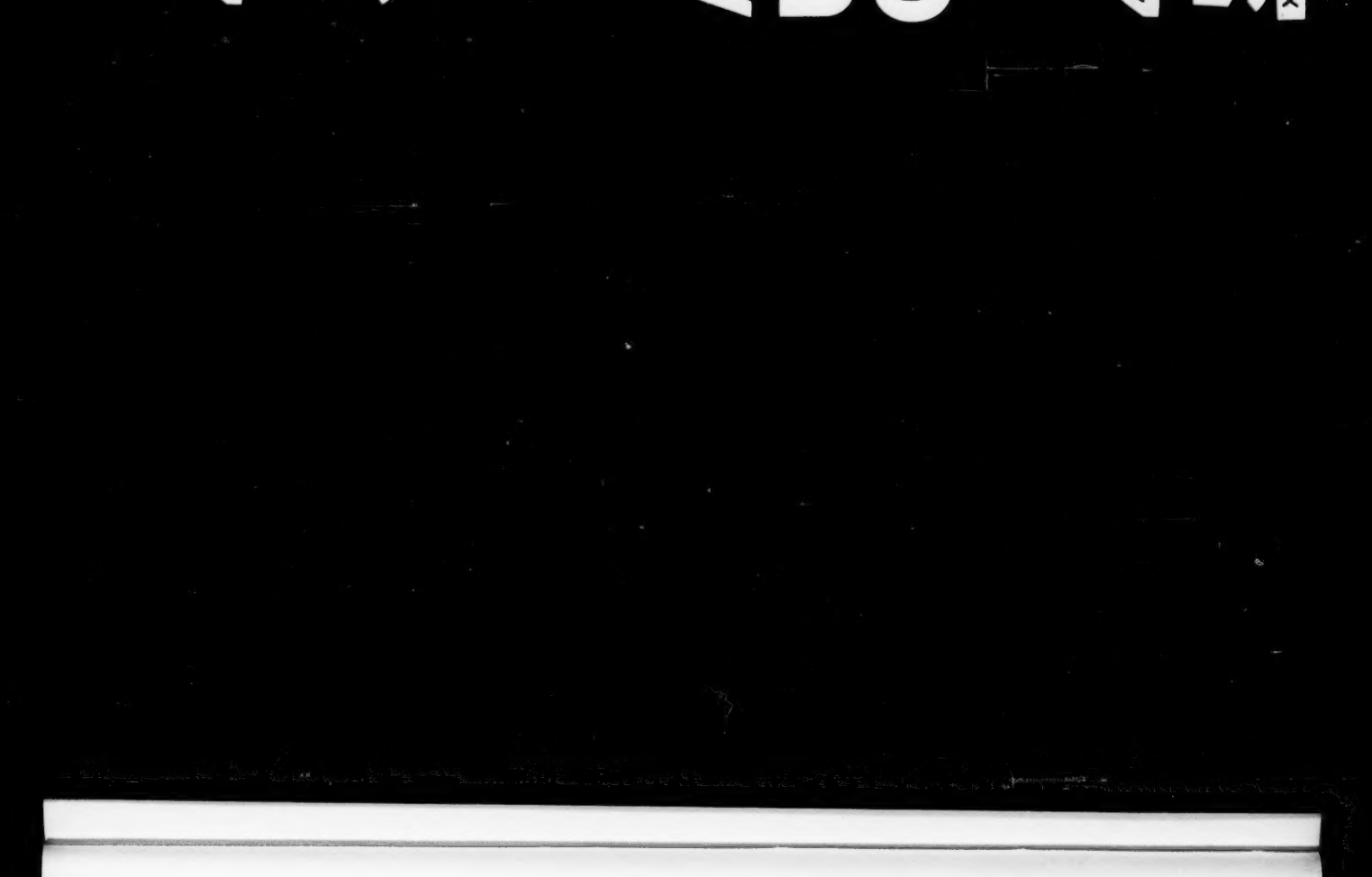
D. M. Mackintosh, of the Kansas State College staff, is now on a European assignment for the War Department, after service in the South Pacific and the Philippines as a captain in the Quartermaster Corps.

E. M. Woods was appointed assistant extension animal husbandman at the University of Missouri, effective January 15, 1945.

Cecil T. Blunn, formerly a member of the staff at the Southwestern Range and Sheep Breeding Laboratory, Fort Wingate, New Mexico, is now with the animal husbandry department at the University of Nebraska.

Fred H. Leinbach is now devoting full time to his duties as head of the animal husbandry department, University of Maryland, after completing an assignment as director of the National Livestock Conservation Program.

J. R. Neale, formerly a member of the animal husbandry staff, University of Wyoming, is now chief of party, Food Supply Mission in Peru, for the Institute of Inter-American Affairs.

Alexander Johnson, who has been in charge of the wool section in WFA office of marketing services in Washington, D. C., has resigned to return to the University of Wyoming.

L. B. Burk, of the grading and market news staff of the Livestock and Meats Branch, WFA, retired from service June 30, 1945, after 27 years of service.